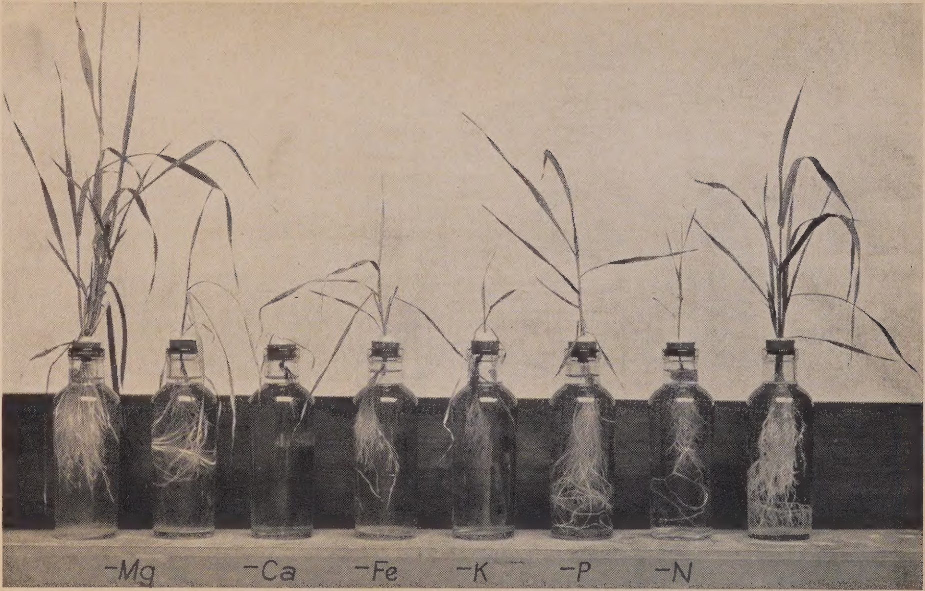
UNIVERSITY OF TORONTO
3 1761 01746572 5

UNIV.OF
TORONTO
LIBRARY



BARLEY PLANTS SIX WEEKS OLD GROWN IN WATER CULTURE

The two end plants received all the essential elements; the others lacked those shown. From a class experiment
Photograph by A. A. Kempin

AN INTRODUCTION TO
PLANT PHYSIOLOGY

By

illiam wen

W. O. JAMES

B.Sc., Ph.D., D.Phil.

Oriel College, Oxford; University Demonstrator
in Botany

263385
16 1 32

OXFORD
AT THE CLARENDON PRESS
1931

1258

OXFORD UNIVERSITY PRESS
AMEN HOUSE, E.C. 4
LONDON EDINBURGH GLASGOW
LEIPZIG NEW YORK TORONTO
MELBOURNE CAPETOWN BOMBAY
CALCUTTA MADRAS SHANGHAI
HUMPHREY MILFORD
PUBLISHER TO THE
UNIVERSITY

PRINTED IN GREAT BRITAIN

PREFACE

THIS book is written for readers of senior school or junior university status, and seeks to give a balanced account of the more elementary aspects of plant physiology. Recent research has not been eschewed, where fundamental principles are involved, but controversial matter has been as far as possible avoided. The aim has been to give an all-round account of the subject as it presents itself to the author, not to fulfil the requirements of any formal syllabus. Nevertheless, while writing was in progress the botany schedules of a number of representative bodies were examined, and found to contain nothing of a physiological nature, which it was not proposed to include in the text.

In compiling the experimental work set out at the end of each chapter the needs of small laboratories of limited means have been kept in mind. Experiments involving special apparatus of an elaborate kind have generally been avoided, being indeed unnecessary for the purpose in view, since a very limited skill in manipulating glass vessels and tubing may be made to accomplish much. The directions given err, perhaps, on the side of too detailed a description, but the success or otherwise of an experiment depends so often upon apparent trifles that the risk has been taken. This part of the book owes much to the co-operation of my wife, formerly botany mistress at Roundhay High School.

The majority of the illustrations are original, where other sources have been drawn upon acknowledgement is made in the text. Those marked with the initials M. S. are from drawings by Miss Marie Solari, B.A., to whom I am greatly indebted.

My sincere thanks are also due to Professor A. G. Tansley, F.R.S., for reading the manuscript, and to Mr. G. R. S. Snow, M.A., for reading that of Chapter VII, and to both of them for making valuable suggestions concerning the subject-matter.

W. O. J.

ISLIP
18th *June* 1931

CONTENTS

Barley plants, six weeks old, grown in water culture . *Frontispiece*

Chapter I. MATERIAL AND ENERGY

page

INTRODUCTION. The nature of plant physiology. Living and not living. The plant and its surroundings . . . 1

THE GREEN PLANT. The plant as a structure. The ability to grow. The green plant and others 6

THE RAW MATERIALS OF GROWTH. Atmospheric carbon dioxide. Carbon dioxide from the soil. Dissolved carbon dioxide. Other sources of carbon 8

PHOTOSYNTHESIS. The diffusion stage. Movements of stomata. Water plants. The action of light. Chlorophyll, physical and chemical properties. The formaldehyde hypothesis. Other internal factors. The interaction of factors. The primary products of photosynthesis. The energy transformed . 13

CHEMOSYNTHESIS. The sulphur bacterium. Iron bacteria. Nitrifying bacteria 32

Chapter II. SUGARS AND THE PLANT MATERIALS FORMED FROM THEM

THE CHEMISTRY OF THE CARBOHYDRATES. Monosaccharides. Disaccharides. Polysaccharides. Glucosides . . . 45

THE CARBOHYDRATES OF THE LEAF. The leaf sugars. The relation between sucrose and hexose. The relation between glucose and fructose. Starch. Glucosides 54

RESERVE SUBSTANCES. Linked reactions 61

THE FATS 64

Chapter III. NITROGENOUS COMPOUNDS

THE CHEMISTRY OF THE PROTEINS. Classification of the proteins. Conjugated proteins 69

Chapter III. NITROGENOUS COMPOUNDS (contd.)

page

THE FORMATION OF PROTEINS AND AMINO-ACIDS IN PLANTS. The synthesis of amino-acids. Protein formation in meristems . 72

NITROGEN ASSIMILATION. Soil nitrogen. Mycorrhiza. Atmospheric nitrogen. The root nodules of the Leguminosae. Nitrogen fixation by other organisms 77

THE NITROGEN CYCLE. Nitrogen fixation in air and soil. Nitrification. The formation of ammonia. Denitrifying bacteria. Oxidizing bacteria. The nitrogen balance of the soil. . 85

Chapter IV. THE DECOMPOSITION OF SUGARS AND THE LIBERATION OF ENERGY

PLANT RESPIRATION. Its nature. Rate. Respiration throughout the life cycle. Respiration and glucose. The action of oxygen. Anaerobic respiration. Aerobic respiration. Biological oxidations. The respiration enzymes. The energy liberated. The respiration of succulents. The respiration of fatty seeds. Respiration in plants and animals 92

ALCOHOLIC FERMENTATION. Zymase 111

OTHER FERMENTATIONS 114

Chapter V. THE PROTOPLAST

THE NATURE OF PROTOPLASM. The visible protoplast. Association. Protoplasmic structure 121

CRYSTALLOID AND COLLOID. Brownian movement. Diffusion and dialysis. Gels. Surface action and adsorption . . 128

THE PHYSICAL STATE OF PROTOPLASM 135

THE CHEMICAL NATURE OF PROTOPLASM 136

ENZYMES. Enzyme catalysis. Extraction and purification. Specificity. Classification 137

MINERAL ELEMENTS. The elements essential for growth. Mineral elements in protoplasm 142

Chapter VI. WATER

page

WATER IN AIR AND SOIL 152

TRANSPIRATION. The site of transpiration. Changes of stomatal opening. Wilting 154

THE UPTAKE OF WATER. Suction pressure. Osmotic pressure. Wall pressure. Suction pressure of root cells . . . 156

THE MOVEMENT OF WATER INSIDE THE PLANT. Passage through the root. Passage through the leaf. The tensile strength of water. The transpiration stream. The path of conduction . . 162

XEROPHYTES. Drought evaders. Succulents. Drought endurers 170

TRANSLOCATION. The penetration of internal membranes. Direction of translocation. Diffusion as a translocation agent. The transpiration stream. Translocation through the phloem 177

THE ENTRY OF SOIL SUBSTANCES. Selection during absorption. Physiological balance. Antagonism 182

Chapter VII. IRRITABILITY

The nature of irritability. The internal sequence . . 193

GEOTROPISM. Geotropic response in primary roots. The clinostat. Geotropic induction in roots. Conduction. Geotropic response in primary stems 196

LIGHT AND PLANTS 204

PHOTONASTY. Periodic movements of flowers and leaves. Changes in the rate of elongation of roots and other organs . . 205

PHOTOTAXIS. Free swimming cells. Chloroplasts . . 208

PHOTOTROPISM. Responses in roots and stems. Coleoptiles. Phototropic induction. Phototropic responses of leaves . 211

Chapter VIII. GROWTH

THE GROWTH OF UNICELLULAR PLANTS. The growth of a colony 222

HIGHER PLANTS. Correlation and inhibition. The life-cycle. Annuals and perennials 224

Chapter VIII. GROWTH (contd.)

page

THE FORMATION OF SEEDS. Dormancy of seeds. Causes . . 226

GERMINATION. The biochemistry of germination . . 229

EXTERNAL CONDITIONS AFFECTING GERMINATION. Oxygen supply. Temperature. Light 235

VEGETATIVE DEVELOPMENT. The measurement of growth. The grand period of growth. Yield. External conditions affecting growth and yield 238

VEGETATIVE REPRODUCTION 244

THE REPRODUCTIVE PHASE 249

LIST OF REAGENTS 254

INDEX 257

I

MATERIAL AND ENERGY

INTRODUCTION

The Nature of Plant Physiology. Since Theophrastus first collected and described the common plants of Greece, mankind has been interested in plant studies in many different ways, and very different points of view have been brought to bear on them. Even before Theophrastus' time, and in all ages since, herbs have been gathered for their real or supposed healing powers, but there has often been but little science in the matter. For a few hundred years now, botanists have studied plants for their own sakes, and have investigated their variety, form, structure, life-processes, and the societies, which individual plants form, without any other motive than the increase of knowledge. Each of these aspects of the knowledge of plants has been given a distinctive name, since each may be a study in itself. The study of life-processes, or functions, and the development which results from them, is called *plant physiology*, and is the subject dealt with in this book.

Divisions such as the foregoing are made for convenience only. It is not possible to isolate a single body of facts, and study them satisfactorily, without reference to any other subject. Natural phenomena are linked together in such complex and often unexpected ways that subjects which might seem at first sight to be quite unrelated have to be considered together. It will easily be realized that plant functions cannot be properly understood unless something is known of the structures with which they are associated, and a knowledge of plant anatomy is, therefore, one of the tools with which the physiologist works. He presses many others into his service as well, and the most important of these are provided by chemistry and physics. Modern developments in these subjects have enabled plant physiologists to push forward their inquiries to an extent which would have been quite impossible before, while, on the other hand, discoveries made by the physiologists have started chemists on new and fruitful lines of investigation; the discovery of osmosis by the botanist de Vries was a case of this kind.

The methods used by plant physiologists at the present day are mainly derived from various branches of chemistry and physics; that is to say, they are similar to the methods employed in the study of non-living matter. The use of these methods has proved extraordinarily fruitful, and our knowledge of plant activities is still increasing so rapidly that it is becoming difficult to keep oneself informed of all the advances which are being made. It might, indeed, seem to an optimistic observer that the use of such methods must eventually reveal to us all the secrets of plant life, but this is not a logical conclusion, though some people believe it, because they like to think that knowledge can be thus unified. Since such a belief implies that living matter is not radically different from non-living, but is subject to the same natural laws and no others, such people may be described as *materialists*. *Vitalists*, on the other hand, are those who feel confident that sooner or later such methods will reach the end of their usefulness, leaving much still unexplained, or, in other words, that living matter is subject to special laws of its own. The strictly scientific attitude, however, is to believe neither of these things, since neither can be shown to be true at present. Meanwhile, whether either belief is held, or none, much may certainly be learnt concerning life processes by physico-chemical methods of inquiry; it is to such methods that the whole of our existing knowledge is due.

Living and Not Living. There is little difficulty in deciding whether common objects are alive or not, but the actual borderline between all living and all non-living things is very difficult to trace. No formal definition can be given which will include all living organisms and exclude all inanimate objects. Nevertheless, matter which we recognize as being alive possesses a number of properties which, taken all together, are characteristic of it, even though each individually may be shared with other things. These properties may be summarized as follows. *Continuous internal change* goes on in protoplasm, or living matter. Even seeds which seem to remain unchanged for years are usually undergoing slow maturation. *Irritability*, or the power to respond to outside changes, is very marked. Some inanimate substances react to particular external effects; selenium, for example, reacts to alterations of light intensity, but none respond to so many influences as protoplasm. The after-effects upon living matter may also be very prolonged. In some seeds exposure to light for only 30 seconds brings them into a suitable condition for germination, when without the light they would not germinate at all. *Growth* by the assimilation of new matter and *reproduction* without outside interference are perhaps the most characteristic features of organic material, though they also can be imitated by the growth of crystals in saturated solutions and the behaviour of emulsions under the influence of external forces.

The Plant and its Surroundings. In spite of their special property of life, plants cannot be studied apart from their environment. Even animals, with their powers of free movement, cannot escape from dependence upon their surroundings; they must feed. In plants, although the connexion is not more real, it is certainly more obvious, because the great majority of plants spend their lives rooted to a single spot. They can only grow if suitable substances enter their roots from the soil, and it is not surprising to find that differences of soil and other surroundings exert easily noticed effects upon them. In a field of wheat, for example, the plants growing in the centre, in the full sun, are short and sturdy; they are bright green before ripening, and finally carry large ears of grain. If the planting has been carried close to a hedge or under the shade of an overhanging oak or elm the stalks run up to an unusual height, the colour of the leaves is much darker, but the ears are smaller; a trained observer, again, can tell a great deal about the soil of a field, and the cultivation it has undergone, by examining the crop growing on it. The state of adjustment which exists between plants and their environment is often very delicate, so that quite small changes of the latter will bring about corresponding changes in the living organism. This is strikingly illustrated by an observation made upon the sensitive leaves of the sunflower (*Helianthus annuus*). Hot, bright weather causes them to lose water rapidly, and as their cells become less turgid their area diminishes. Such shrinkages and corresponding recoveries are very rapid and so easily brought about that it has been found possible to measure the leaf's changes as clouds pass across the sun.

The plant is not entirely the creature of its surroundings, however. High winds, shallow soil, and lack of soil-water will convert a pine-tree growing on the side of a chalk-down into a stunted little bush, but no adversity of environment would change it into a juniper. Regarded in this way plants may be said to be compounded from two sources, the seed, or spore, and the environment; and their physiology to consist of the results produced by external influences working upon materials provided by the plant's inheritance.

The influence between plant and environment is mutual.

Not only do the natural surroundings mould the plant, but they are themselves liable to be changed by it. Low-lying land, moist enough for Sphagnum moss to become established upon it, may become covered with a thick layer of the plant, which grows at the tip as it dies away below. The mat of dead material blocks all drainage, and may eventually retain water over a wide area of land, turning it finally into a deep bog. The roots or underground stems of many plants growing in loose or sandy soils bind the particles together, and so prevent erosion by sea or wind. Marram grass (*Psamma arenaria*) growing on sand dunes, fixes them against the action of wind, while *Spartina Townsendii* has proved a valuable agent in reclaiming tidal salt mud from the sea. These are a few instances of the many ways in which plants alter the physical features of a countryside, and they may also cause considerable changes in the atmosphere above. A covering of vegetation, for example, greatly reduces the temperature of the earth's surface and the lower layers of the atmosphere, owing to the evaporation constantly going on from the leaves. An entirely different case is that of yeast, a unicellular plant, normally producing large quantities of alcohol, which pass out into the liquid in which the cells are growing, and so become part of their environment. From 4 to 10 per cent. of alcohol is fatal to most submerged organisms, but yeast is not injured at these concentrations, though if the medium is limited sufficient alcohol will accumulate to kill it in the end.

In studying the physiology of plants we are primarily concerned with things going on inside the plants themselves, but it will be clear that we also have to consider the ways in which these happenings depend on circumstances outside. In other words, we always have to take environment into account, the connexion between internal and external events being so close that no real separation can be made.

The Plant as a Structure. All material structures, whether living, such as the plant, or non-living, such as a machine, can only be formed from stuff already in existence. The appearance and nature of the *raw materials* may be very much changed in the process, but there is at the end just as much matter by weight in the final product as there was in the original substances. To construct a machine there is also a second necessity; there must be *energy* to shape the materials and fasten them together in the required ways, and finally, when the machine is complete, further energy must be supplied to drive it. This is not necessarily all of one kind, there is the mental energy of the designer and the muscular energy of the manufacturing engineers, besides electrical and other forms which they may have turned to their purpose. Finally there is the explosive or other energy which is used to drive it. Similarly the formation of a plant from inorganic materials can only go on when energy is brought to bear upon them, and the plant's life can only continue if there is a certain amount continuously available. The essential requirements, material and energy, for formation and maintenance are thus the same, both for the living and non-living.

The Ability to Grow. One of the most important ways in which the living structure differs from the non-living lies in its ability to grow. Machines are formed by various agencies, usually under human control, and once formed remain unchanged except for wear and tear. Plants, on the other hand, have a steady development and are subject to continuous change throughout their existence. Each individual has a definite history, called its *life-cycle*, which is repeated more or less closely by its successors. More important still, once the cycle is initiated by the formation of a spore or seed it continues automatically to its close without human interference. In this sense the structure manufactures itself. It is constructed, that is to say, by the play of natural forces, those which we have named the hereditary tendencies of the seed, together with the forces of the environment. This spontaneous development of the living organism is called its *growth*, and the fashion in which matter and energy are assimilated into the plant body in growth is one of the most important studies of plant physiology. It forms the subject of this chapter.

The Green Plant and Others. The green plant is unique in the way in which energy and material are built into it. The raw material which it uses is entirely inorganic, and in this it differs sharply from animals, which depend entirely upon products originally formed by the green plant for their growth and maintenance. Colourless plants resemble animals in this respect, since they obtain their materials from the same source, and are called *heterotrophic* to distinguish them from the self-supporting green plants, which are called *autotrophic*. The green plant is thus in plain fact the mainstay of the organic world. The only other organisms which synthesize organic matter from inorganic sources are a few obscure bacteria, and many of these do so only when there is no previously formed organic matter in their immediate surroundings. The green plant is, therefore, of special importance as the principal system which transfers matter and energy from the inorganic to the organic world.

The variations of structure and function in the entire range of green plants is very wide, but in the essential features of the assimilating process they resemble one another to a remarkable degree. The sources of inorganic material are few and almost always the same, and the energy drawn upon is invariably direct radiation from the sun. The essential property which distinguishes green from other plants and gives them their unique powers is the presence of the mixture of pigments called *chlorophyll.* The individual pigments are identical in their chemical composition in all plants which possess them, and the proportions in which they are mixed vary only to a moderate degree.

THE RAW MATERIALS OF GROWTH

Ten elements, carbon, hydrogen, oxygen, nitrogen, potassium, calcium, magnesium, iron, phosphorus, and sulphur, are essential to the growth of the great majority of plants, and are derived in various compounds from the surrounding air and soil. Of the solid material in the plant remaining after all water has been driven off, about 90 per cent. is composed of compounds containing only carbon, oxygen, and hydrogen. On this account these three elements are of particular importance, but their significance is still further increased by the fact that the assimilation of energy is associated exclusively with their entrance into the plant. The assimilation of the seven remaining elements has little or no association of this kind. Carbon dioxide from the air and water from the soil are the raw materials in *carbon assimilation.* The alternative name of the process, *photosynthesis*, denotes that light energy is also involved.

Atmospheric Carbon Dioxide. The atmosphere surrounding plants contains only a very low percentage of carbon dioxide, much lower, for example, than its percentage of the inert gas argon. The average proportion in the lower atmosphere is about three parts in ten thousand, but this decreases at higher levels. Even near the earth's surface the proportion is anything but constant, and there are considerable daily and seasonal variations. It has been shown, for instance, that by day the value is usually about 12 per cent. lower than at night. This is mainly due to the removal of carbon dioxide by assimilating plants, which ceases at nightfall, and measurements made in mid-Atlantic showed no fluctuations of this kind.

It has been calculated that vegetation assimilates about 1/35th of the atmosphere's carbon dioxide each year, but in spite of this fact the percentage has not altered appreciably during the past century. It is clear, therefore, that there must be agencies which release carbon dioxide into the atmosphere. The principal of these are the activity of volcanoes, combustion, and the respiration of plants and animals, including the so-called 'soil respiration', due to respiration and fermentation by organisms in the surface layers of the soil. As a result of all these changes carbon is kept in continuous circulation and its principal transformations are shown in fig. 1.

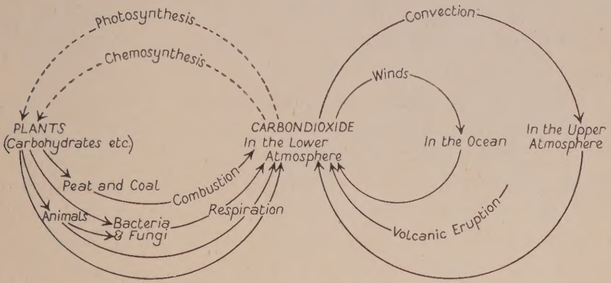

Photosynthesis
Chemosynthesis
PLANTS
(Carbohydrates etc)
Peat and Coal
Animals
Bacteria
& Fungi
Combustion
Respiration
CARBONDIOXIDE
In the Lower
Atmosphere
Convection
Winds
In the Ocean
In the Upper
Atmosphere
Volcanic Eruption

FIG. 1. THE CARBON CYCLE

Diagram to show the principal natural transformations and movements of the element. On the left-hand side the broken lines indicate endothermic reactions (energy taken in), and the continuous lines exothermic reactions (energy set free)

These various agents operate in different places and to some extent at different times. Winds and convection currents, especially over hot places, bring about a certain amount of mixing, but it is by comparison a slow process and local differences of concentration are continually being set up. During an eruption in Guadeloupe the carbon dioxide content of the neighbouring atmosphere rose to 2 per cent., and measurements made in the centre of London on a bank holiday, when factory fires and human respiration would be much less than usual, showed a carbon dioxide content of about half the normal value. These are perhaps extreme examples, but they show that there are likely to be considerable fluctuations in the amount of carbon dioxide available for any given set of plants.

Combustion, human respiration, and volcanic eruption occur mostly at a distance from areas covered by vegetation, so that the air of such districts usually holds a comparatively small amount of carbon dioxide. At a distance from the above sources the carbon dioxide produced by the respiration of the plants themselves, by grazing animals, and by the organisms of the soil are of great importance.

Carbon Dioxide from the Soil. The soil population consists of many unicellular plants and animals, but by far the most numerous and important are the bacteria. When plant or animal remains fall upon the ground or are buried in it, various bacteria break down their materials, and carbohydrates, such as starch, cellulose, &c., which were present in the original material, are split up and oxidized, eventually giving rise to gaseous carbon dioxide. These changes are carried out in many stages, and often many different organisms take part. Free oxygen is not always necessary, since *anaerobic bacteria*, so named because they are able to exist in an atmosphere which is entirely devoid of gaseous oxygen, can cause changes of oxygen distribution among the organic compounds themselves. In this way oxidized and reduced substances are formed at the same time in the process which is called *fermentation.* When cellulose, which is very abundant in plant remains, is fermented or decomposed in the absence of oxygen, a number of organic acids, such as formic, acetic, and butyric, are produced. These contain a relatively high amount of oxygen, but hydrogen and methane appear simultaneously. In the presence of free oxygen, methane and the acids are completely oxidized to carbon dioxide and water, a different organism being concerned in each case. The hydrogen also becomes oxidized to water, so combustible gases are never given off by ordinary soils.

The changes going on are thus very complex, since many substances are being acted upon by a host of bacteria, fungi, and other organisms. Of the gaseous carbon dioxide which is always being formed, a great deal escapes into the lower layers of the air. For example, an unmanured and fallow field was found to give off about 250 c.c. of free carbon dioxide per hour for every square metre of its surface. The carbon dioxide thus produced by the soil is liberated near the position of carbon dioxide removal, i.e. near the leaves of plants growing upon it, and so tends to prevent the concentration from falling.

The process has been increased artificially by what is called 'carbon dioxide manuring'. Fermenting manures are added to the soil in order to increase its output of carbon dioxide. Near towns an attempt has also been made to utilize waste furnace gases, which are very rich in carbon dioxide, by laying pipes to deliver them over the surface of farm fields. In favourable years, when light and warmth have been sufficient, increased harvests have been secured by these means.

Dissolved Carbon Dioxide. Natural waters contain a great deal of carbon dioxide, but only about one fiftieth of it is dissolved gas. The rest is associated with calcium and other bases as carbonates, bicarbonates, and the ions which arise from them, by far the most plentiful of these forms being the bicarbonate ion $-HCO_3$. Such a solution is said to be 'buffered' in respect to carbon dioxide, since whenever pure carbon dioxide is removed it is at once replaced from the stores present in its compounds. An equilibrium is set up between the combined and free carbon dioxide in solution, and again with the gaseous carbon dioxide of the overlying atmosphere. If the latter is increased some of it goes into solution, in which one fiftieth part of the carbon dioxide remains as dissolved gas, and the remainder passes into the combined forms. Conversely, if the atmospheric pressure of carbon dioxide is reduced free gas is given off from solution, the bulk of it being derived from the dissolved compounds. The oceans contain enormous quantities of such substances and therefore act as regulators of the carbon dioxide in the atmosphere above them. These adjustments are very slow, however, and do not prevent minor alterations occurring in the atmosphere from day to day.

Other Sources of Carbon. Enormous quantities of carbon exist as the bicarbonate ion $-HCO_3$, and both fresh and salt water contain a great deal more of this ion than they do of dissolved carbon dioxide. Various opinions have been held as to whether submerged plants are able to assimilate this source of carbon direct, but it is now believed that under most conditions they are quite unable to do so. There are also other possible sources of carbon supply to the plant, such, for example, as the *humus*, or decayed vegetable matter, which is reached by roots penetrating the soil. It was originally believed that carbon compounds from this source were the green plant's main supply, but it has since been shown that this is not the case. It appears, in fact, that the sole source of carbon for the typical green plant consists of carbon dioxide. Land plants depend upon the gaseous carbon dioxide of the atmosphere, while water plants assimilate dissolved carbon dioxide, but not its derivatives.

PHOTOSYNTHESIS

By examining a leaf section it can readily be seen that the green pigment, which is essential for the assimilation of carbon, is limited to certain plastids or small bodies differentiated from the general cytoplasm (Chapter V). These are called *chloroplasts* and occur in the cortical cells of green stems and aerial roots as well as in the inner tissues of leaves. Photosynthesis goes on only in the chloroplasts, so their distribution is a matter of importance.

Fig. 2 (p. 15) shows the position of the chloroplasts in the leaf of a land plant, and fig. 6 their position in the submerged ribbon-shaped leaf of *Sagittaria sagittifolia.* In the land plant chloroplasts are most abundant in the elongated cells of the *palisade tissue* (fig. 2, *a*), but they also occur in the more scattered cells of the *spongy mesophyll* (*b*). There are no chloroplasts in the outermost layer of cells, or *epidermis*, except in the *guard cells* of stomata (*c*). The cells which contain the chloroplasts are always vacuolated, and the plastids occur in the thin layer of cytoplasm lining the cell wall. The walls are thin and are composed of cellulose; they are never lignified or suberized.

The Diffusion Stage. In many leaves the stomata are confined entirely to the lower surface, and advantage has been taken of this fact to show that carbon dioxide enters the leaf only through their open pores. A small glass chamber was fitted to each surface of a leaf and a separate stream of air passed through each. The air which was passed through the upper chamber did not lose any of its carbon dioxide, but the stream passing through the lower chamber did. For another experiment the water plantain (*Alisma plantago*) was used. This has stomata on both surfaces of its leaves, the upper surface having 135 stomata for every 100 on the lower. When the experiment was carried out with this leaf it was found that the upper gas-stream lost 146 parts of carbon dioxide for every 100 lost by the lower. In other words, the amount of carbon dioxide which entered the leaf was approximately proportional to the number of stomata, and it seems clear from these experiments that, in the main, carbon dioxide gets into the leaf through the stomatal openings. A certain amount may penetrate the epidermal cells, but in the ordinary way this is quite negligible.

A sunflower (*Helianthus annuus*) leaf in bright light assimilates about 13 c.c. of carbon dioxide per hour for every 100 sq. cm. of its surface. Only about one per cent. of this area is occupied by stomatal openings (fig. 3), and at first sight it seems surprising that the low proportion of carbon dioxide in the atmosphere is sufficient to ensure such a supply. A careful examination of the diffusion of gases through small openings provided the solution of the mystery. It was found that a single large opening in a surface does not let nearly as much gas diffuse through it as a number of small openings set at a few diameters apart, and having the same total area. Following up this observation, the number, size, and distribution of the stomata on sunflower leaves were examined. From the figures obtained it was calculated that no less than 258 c.c. of carbon dioxide might diffuse each hour through every 100 sq. cm. of leaf surface under normal atmospheric conditions. This is enormously in excess of the highest value observed for this species, which is only about 15 c.c., and it is, therefore, evident that it is not the smallness of the stomatal apertures which restricts photosynthesis.

In the air spaces inside the leaf there are no air movements or draughts, and the carbon dioxide travels only by diffusion. This is a physical process depending upon the

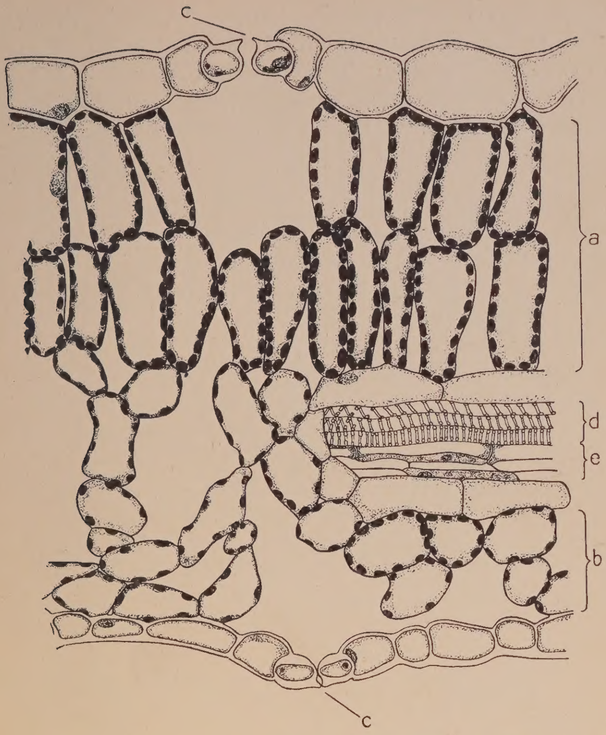


Fig. 2. VERTICAL SECTION OF A SMALL PIECE OF RHUBARB LEAF (*Rheum officinale*)

a. palisade; *b*. spongy mesophyll; *c*. stomata, which in this leaf occur on both surfaces; *d*. xylem of a small vascular bundle; *e*. phloem. Chloroplasts shown in solid black. Highly magnified spontaneous movements of molecules. The molecules of all gases and of all substances in solution are supposed to be in continuous motion. They travel in straight lines until they collide with other molecules, when their course is changed, but their velocity is supposed to be unaltered.

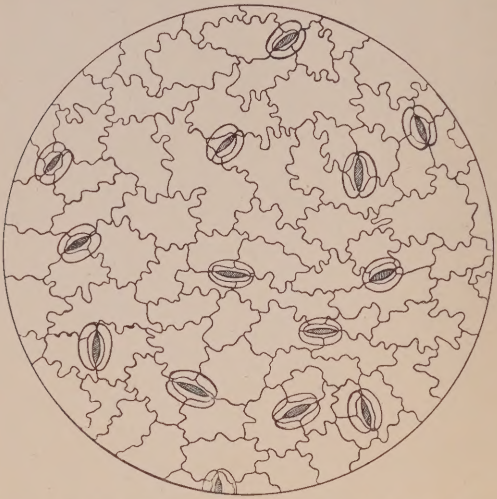


FIG. 3. Surface view of the epidermis of *Hypericum calycinum* with stomatal pores shaded. Highly magnified. (M.S.)

In any volume of gas the contained molecules are moving in all directions at random, and if, for example, two equal volumes containing different numbers of molecules are in connexion with one another, the final result of the haphazard movements will be to equalize the two concentrations. This is called diffusion and takes place because there will be more frequent invasions from the more numerous molecules into the less numerous than vice versa. It is important to notice that the pressure, i.e.

number of molecules, of other gases present will not affect this equalizing tendency, or, in other words, each gas diffuses according to its own pressure differences, and is not affected by those of other gases. In solutions the situation is similar, but diffusion is slower because of the obstacles afforded by the closely packed molecules of the solvent.

The cellulose walls of the cells surrounding the air spaces of the mesophyll are impregnated with water, indeed in the natural condition water accounts for the greater part of their weight. When carbon dioxide comes into contact with these wet surfaces it goes into solution, and the final stages of the journey to the chloroplast is performed by diffusion in a watery medium. Gaseous diffusion is a very slow process: if an opening to the atmosphere one square centimetre in area was made one centimetre above a surface of caustic soda, only 0·003 c.c. of carbon dioxide would enter the absorbent in an hour. Diffusion through water is much slower still, because the molecules of the gas meet with much more resistance to their free movement. The distance which lies between the stomata and the chloroplasts is, however, very short, and in land plants the time taken by the gas in the diffusion stages is not very long.

Movements of Stomata. It is well known that the guard cells of stomata alter their shape from time to time, and so change the area of the opening between them. A partial closing of the stomata might be expected to hinder the diffusion of carbon dioxide into the leaf and so slow down the rate of assimilation. It has been shown that this actually does occur, and hence in studying the rate of photosynthesis it is essential to know the condition of the stomata.

The behaviour of stomata is very complex. In many plants such as maize and other cereals they only open for a comparatively short period of the day, whereas in the potato plant they remain open except for a short period at night (fig. 4), and in *Hydrangea* they are said to remain open all the time. Their period of opening is altered to some extent by external conditions, and the most important of these is light. Most stomata open fully in bright light and close in darkness.

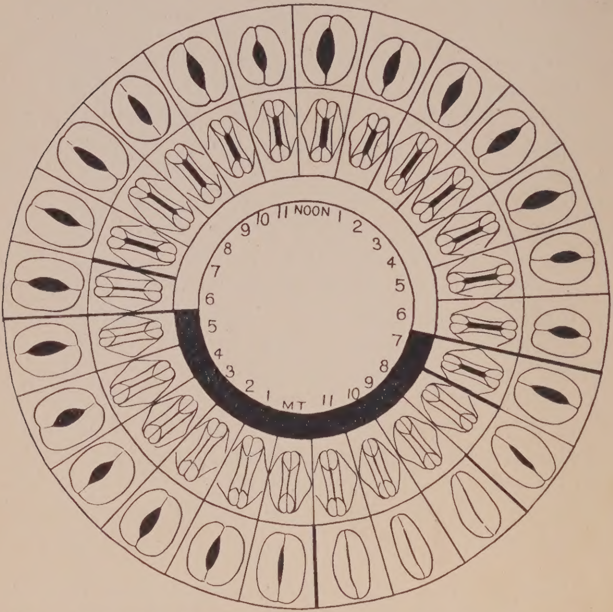


FIG. 4. DIAGRAM TO SHOW THE DAILY OPENING AND CLOSING OF STOMATA
Inner circle maize; outer circle lower surface of potato. Hours of darkness are shown by the black band. (After Loftfield, modified. M.S.)

These movements are apparently brought about by changes of osmotic pressure in the guard cells, which contain chloroplasts, and are consequently able to form sugars when illuminated. It has been noticed that starch granules, previously present in the guard cells, disappear at the same time, giving rise to further sugar. Under these conditions osmotic pressures are developed, which are much higher than those of the surrounding cells. Water passes into the guard cells, which swell considerably in consequence. The effect of the swelling is not to close the stomatal aperture, as might be supposed, but actually to enlarge it. Having been distended with water the guard cells are rigid like two sausage-shaped balloons blown up with air, and they stretch their walls to their fullest extent. They then appear as shown on the right-hand side of fig. 5. When water is withdrawn, owing to the reconver-

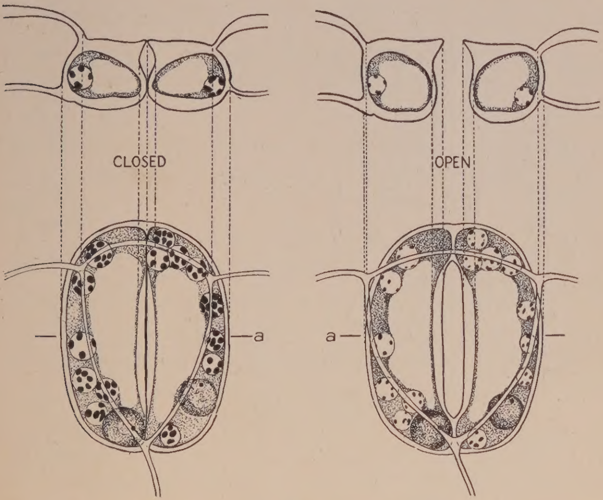


FIG. 5. STOMA OF *Hypericum Calycinum*

On left-hand side the stoma is shown closed and on the right open. Surface views below, with sections along the line a a above. Chloroplasts are left white and their starch grains shown in solid black. Highly magnified sion of sugars into starch and its escape into other cells, the guard cells lose their rigidity and sag together as the balloons would when deflated. (*See* fig. 5.)

Water Plants. The movement of carbon dioxide towards the chloroplasts of submerged plants is simpler in some respects than the corresponding movement in aerial plants. The carbon dioxide is in solution at all stages and the leaf structure is usually simpler also. A typical example is shown in fig. 6. There are no stomata, and the dissolved gas diffuses through the epidermis, which has thin and unmodified outer walls. The path of diffusion inside the leaf is much shorter than in aerial leaves, but diffusion is so slow in solution that it may still retard the rate of photosynthesis considerably. If the plant were submerged in a solution of carbon dioxide alone diffusion through the water layers outside would also cause a delay, and under these conditions water currents would play a great part

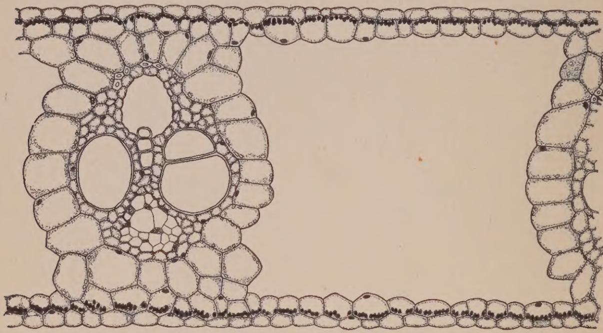


FIG. 6. TRANSVERSE SECTION OF PART OF A SUBMERGED RIBBON LEAF OF *Sagittaria Sagittifolia*

The chloroplasts are shown in solid black. A large air space appears between the two vascular bundles. Such air spaces appear in many submerged leaves, and accumulate carbon dioxide from respiration as well as other gases. Highly magnified. (M.S.)

as accelerators of the process, because they would continually bring fresh carbon dioxide to the plant. Actually, however, most natural waters, and especially 'hard' ones, contain a good deal of carbonates and bicarbonates, and only about a fiftieth of their carbon dioxide supply is ever present as dissolved gas. The result is that as plants remove carbon dioxide it is spontaneously replaced from the bicarbonates owing to the chemical equilibrium which exists in solution. (*See* p. 11.) When a molecule of calcium bicarbonate releases a molecule of carbon dioxide to maintain this equilibrium, it also forms a molecule of the normal carbonate.

$$Ca(HCO_3)_2 \rightarrow CaCO_3 + CO_2 + H_2O$$

This is why the surfaces of plants growing in hard water often develop an incrustation of chalk as carbon dioxide is removed by their assimilation. The break-down of bicarbonate goes on rapidly at the point where carbon dioxide is removed, so that the retarding influence of diffusion is not felt, and the rate of assimilation remains fast.

The Action of Light. Some chemical reactions, such as the liberation of free iodine from a solution of potassium iodide, only go on in the presence of light. During their course light energy is absorbed, and either rendered latent, or converted to some other form such as heat. They are called *photochemical reactions*, and carbon assimilation by plants is a complex instance of this sort. Unfortunately, the part played by light in any photochemical reaction is not yet properly understood, and consequently a complete explanation of the action of light in photosynthesis cannot be given. Assimilation is not, moreover, a single reaction, and not all its stages are affected by light. The rate of a simple photochemical reaction is unaffected by a change of temperature, but it has been shown that if light and carbon dioxide are abundant, the rate of photosynthesis is about doubled when the temperature rises from 0° C. to 10° C. This increase is brought about by the effect of the temperature rise upon chemical reactions, which are not sensitive to light, but which belong to the series of reactions necessary for carbon assimilation.

Sunlight is not a single form of energy but a mixture of radiations of various kinds, distinguished by their different wave-lengths. White light can be analysed into its constituents and the resulting coloured lights then contain energy of one wave-length only, or rather a mixture of a few closely related wave-lengths. The power to bring about photosynthesis is not limited to any one wave-length, but is possessed by the whole visible spectrum, i.e. all the radiations to which the human eye is sensitive, and by some others as well. These radiations are not all equally efficient for the purpose, however, and according to recent measurements the relative efficiencies of different coloured lights are roughly Blue 3, Green 4, Yellow 5, Red 5·4.

It should be noticed that green light is able to bring about photosynthesis, though actually it can play little part in the process, because it is nearly all reflected from the chloroplast surfaces. Chlorophyll cannot, therefore, be regarded as a perfect light absorber for the purpose of photosynthesis, and it has been suggested that some plants develop colours complementary to the principal rays which fall upon them, with the result that light absorption is improved. The special pigments of the red and brown seaweeds have been ascribed to a 'chromatic adaptation' of this kind. It seems probable that certain lowly organisms, such as the blue-green alga *Phormidium foveolarum*, do behave in such a manner, but there is much less evidence that the seaweeds and higher plants do so as well.

Chlorophyll. Physical and Chemical Properties. Chlorophyll is easily shown to be one of the essential parts of the photosynthetic system (**exp. 42**), and its importance depends upon two factors, its power of absorbing light and its chemical characters. Most plant tissues are colourless and more or less translucent, and therefore absorb only a small proportion of the light which falls upon them. Chlorophyll, on the other hand, absorbs a large proportion of the light which strikes it, and this energy is not merely retained by the pigment, but becomes involved in a complex series of changes in the course of which carbon dioxide is reduced and sugars formed.

When sunlight falls upon a leaf about 70 per cent. of it is absorbed, the rest either passes right through or is reflected from the shiny cuticle. Of the 70 per cent. absorbed the chlorophyll probably takes up no more than 4 per cent., while the remaining 66 per cent. goes into the colourless tissues, and is probably of no direct importance in photosynthesis. Chlorophyll absorbs very little green light, but takes up other wave-lengths to a greater extent. When the light which has passed through a chlorophyll solution is analysed into its colours by a prism, it is seen that certain wave-lengths are missing. These of course are the radiations absorbed by the chlorophyll solution. Fig. 7 B represents diagrammatically the absorption spectrum of chlorophyll, and it will be seen that the principal bands, or regions of absorption, lie among the red and blue rays, both of which are able to cause photosynthesis, though to different extents.

Sunlight as it arrives at the earth's surface contains different intensities of the different radiations. Fig. 7 A shows the distribution of energy in the normal spectrum, but there are minor fluctuations according to conditions. The light which arrives at evening, for instance, has less blue light, i.e. less energy of the shorter wave-lengths, than morning light, and consequently appears redder. This is because the slanting rays pass through a greater length of the atmosphere which absorbs blue light more than red. Water, on the other hand, absorbs long wave-lengths more than short, and light which penetrates into the sea or other natural waters loses its red components. Since photosynthesis is brought about by any visible radiation it continues under all these changing conditions, though its rate may vary according to the intensity and efficiency of the incident rays.

Chlorophyll can be extracted from leaves, without changing its composition, by a mixture of 80 per cent. acetone and 20 per cent. water, and can afterwards be separated from various impurities by transferring it to petrol ether. It forms a rich green solution with a blood-red fluorescence. It is not a pure substance, but has been shown to be a mixture of four pigments, which have been given the names *chlorophyll a*, *chlorophyll b*, *carotin*, *xanthophyll.*

The first two are green, the former being rather bluer than the latter. Carotin gives an orange-red solution, and xanthophyll a yellow. The four substances can be separated from one another by means of their different solubilities in various organic solvents. For instance, xanthophyll is more soluble in methyl alcohol than the other pigments, and can be separated from a petrol-ether solution of the mixed substances by means of it. Carotin is soluble in pure petrol ether, but the green pigments only dissolve in it if traces of methyl alcohol or acetone are present. If the latter are removed by washing with water, chlorophyll *a* and *b* are precipitated, while carotin remains in solution alone. The two chlorophylls can be separated by shaking them up with petrol ether and 90 per cent. methyl alcohol. When the two solvents separate, chlorophyll *a* goes with the petrol ether which retains a little of the alcohol, and chlorophyll *b* with the methyl alcohol itself.

Chlorophyll can be completely extracted from leaves by boiling them with ethyl alcohol, but in this case the green pigments undergo a change. A reaction occurs between chlorophyll and the alcohol, with the formation of a substance, ethyl chlorophyllide, which is not identical with true chlorophyll. Chlorophyll *a* and *b* are closely related in their molecular structures, and contain the elements carbon, hydrogen, oxygen, and nitrogen in various complex groupings and also a small proportion of the metal magnesium. Carotin is a complex hydrocarbon, and xanthophyll is closely related to it, but contains a small amount of oxygen in its molecule. An important property of the

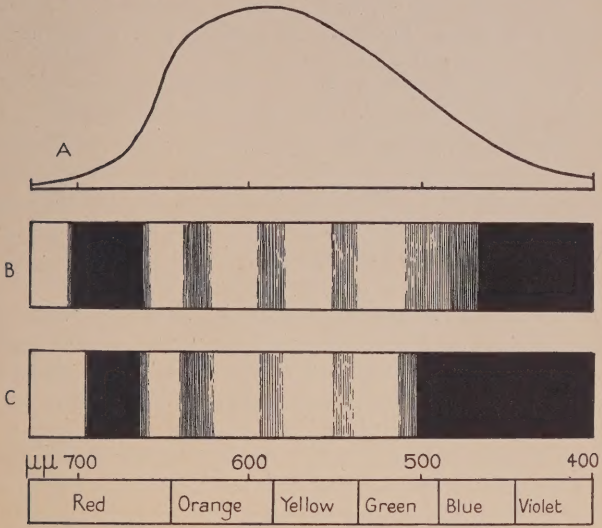


FIG. 7

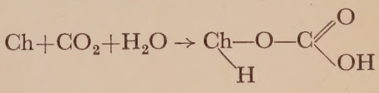
A. Approximate distribution of energy in the solar spectrum; subject to considerable fluctuation
B. Absorption spectrum of a colloidal solution of chlorophyll
C. Absorption spectrum of a stinging nettle leaf
(*B* and *C* after Willstätter)

extracted green pigments is their reaction with acids which remove magnesium from the chlorophyll molecules to form magnesium salts. The chlorophyll residue changes to an olive-green colour, and is called *phaeophytin.* Carbon dioxide in water, i.e. carbonic acid, is able to act upon chlorophyll in this way.

It is not likely that the activity of chlorophyll in photosynthesis comes to an end with the absorption of light, and various attempts have been made to discover any further part it may play in the process. It has been found, for example, that the amount of chlorophyll in a leaf remains practically the same whether photosynthesis is going on or not, and that the proportions of the four pigments also remain unaffected. It is clear, therefore, that none of them are used up in the process, and if they enter into combination with the carbon dioxide, they must be released again in the same form. Various suggestions have been made as to the part they play, but the one with the most experimental evidence to support it is as follows.

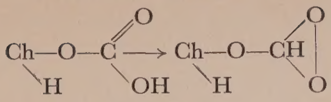
Carbon dioxide and water are supposed to react with the magnesium in chlorophyll *a* and *b*, but phaeophytin and magnesium carbonate are not formed, because the reaction stops short at an intermediate compound in which carbonic acid is added on to the chlorophyll molecule.

Putting Ch for chlorophyll, this step may be represented

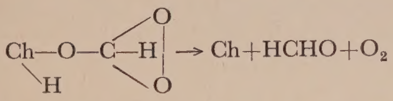


$$\mathrm{Ch+CO_2+H_2O \rightarrow \underset{\diagdown H}{Ch}-O-C}\begin{matrix}\diagup\!\!\diagup\,\mathrm{O}\\ \diagdown\,\mathrm{OH}\end{matrix}$$

The interaction of carbonic acid and chlorophyll outside the plant will go on in the dark, so there is no reason to believe that this stage is the photochemical one. It is suggested, on the contrary, that when the chlorophyll-carbonic acid compound has been formed light causes rearrangements inside the complex molecule as follows:



The final arrangement gives a substance, called *chlorophyll formaldehyde peroxide*, which contains more energy than the first. The compound is then said to be activated, and is in a peroxide form, that is to say, it readily loses oxygen. When this happens the carbon dioxide is reduced to formaldehyde, and the original chlorophyll is released.



If six molecules of formaldehyde unite together, or *polymerize*, the result is a molecule of hexose sugar, the first easily recognized product of photosynthesis.

$$6HCHO \rightarrow C_6H_{12}O_6$$

The Formaldehyde Hypothesis. Nearly all the theories which have been invented about the intermediate stages of photosynthesis have supposed formaldehyde to be formed. This is because chemists have shown that under the influence of strong alkalis, formaldehyde does produce a mixture of sugars, the bulk of which are hexose sugars such as occur in plants. The presence of formaldehyde in assimilating leaves has never been clearly established, however, and attempts to 'feed' plants with it artificially have also led to uncertain results. The production of formaldehyde from carbon dioxide and water, with the help of light, has also proved very difficult to bring about, and at the present time there is but slender evidence that it occurs in plants. There is, on the other hand, no known substance more likely to occur at this stage of the process, and definite proofs of its presence may eventually be forthcoming.

Other Internal Factors. Attempts have been made to synthesize sugars by bubbling carbon dioxide through chlorophyll solutions outside the plant. They have all failed under whatever conditions they were carried out, because chlorophyll is not the only part of the chloroplast which is engaged in the process. This has been shown by measuring the photosynthesis of young bean seedlings from the time they were a week old. At this period they were fully green, but could hardly assimilate at all. After a fortnight, however, assimilation was rapid, though there was no more chlorophyll than before. The increased photosynthesis must have been due to the development of some other factor, often referred to as the 'internal factor', in the chloroplasts, whose formation in the bean plant is slower than that of chlorophyll. The nature of this factor is still unknown.

The growth of the plant depends upon an adequate supply of certain inorganic substances (*see* p. 142), and any deficiency of these has a very marked effect upon photosynthesis. Magnesium, for example, is necessary for the formation of chlorophyll, but other essential elements, such as phosphorus, do not enter into any of the known parts of the photosynthetic apparatus. The action of such substances as these is very obscure.

Water is also essential for photosynthesis, and is derived from the internal supply which reaches the leaves from the roots. It is always present in very considerable excess, the quantity of water used in photosynthesis being a very small proportion of that which passes through the plant.

The Interaction of Factors. The speed at which photosynthesis goes on depends upon the concentration of available carbon dioxide, the quantity of chlorophyll, the intensity of light, temperature, and other factors. Each of these exercises its effect upon the rate of the process, and an increase in any one of them may cause a faster rate. An increase of carbon dioxide concentration from 0·1 per cent. to 0·2 per cent. was found to accelerate the rate of photosynthesis of the water moss *Fontinalis antipyretica* by 42 per cent. An increase from 0·3 per cent. to 0·4 per cent. only caused an additional 35 per cent., while an increase from 3 per cent. to 4 per cent. caused no acceleration at all. If a brighter light was used, there was a greater response to increases in the concentration of carbon dioxide. Two things have to be remembered, therefore: first that the effect of an increase of any given factor is greatest when its intensity is low, and second that its effect is always modified by that of other factors. The interference of other factors can, however, be reduced to negligible proportions by making their intensities so high that further increases do not accelerate photosynthesis. In this way the effect of single factors can be isolated, and studied in detail.

If all the controllable factors are increased, photosynthesis can be made to go on much faster than under normal outdoor conditions. There is a limit, however, above which the mechanism breaks down; too high a light intensity destroys the chlorophyll by oxidation, and too much carbon dioxide acts as an anaesthetic.

The Primary Products of Photosynthesis. The first clearly recognizable products of photosynthesis are the sugars, glucose, fructose, and sucrose, and in all probability the last is derived from the first two. Glucose in plants readily gives rise to starch, which is easily detected (*see* **exp. 4, etc.**), and can be seen as granules in assimilating chloroplasts (fig. 8). All the chemical changes of the assimilatory process go on therefore in the chloroplasts themselves, since the carbon dioxide remains unchanged until it comes in contact with their surfaces. Sugar and starch contain less oxygen than the carbon dioxide and water necessary to form them, and the oxygen eliminated during the process is set free in gaseous form. In this condition it is given off into the intercellular spaces of the leaf, and diffuses out through the stomata to the atmosphere. In submerged plants the gas may go into solution in the surrounding water, but if it is given off rapidly, or if the water

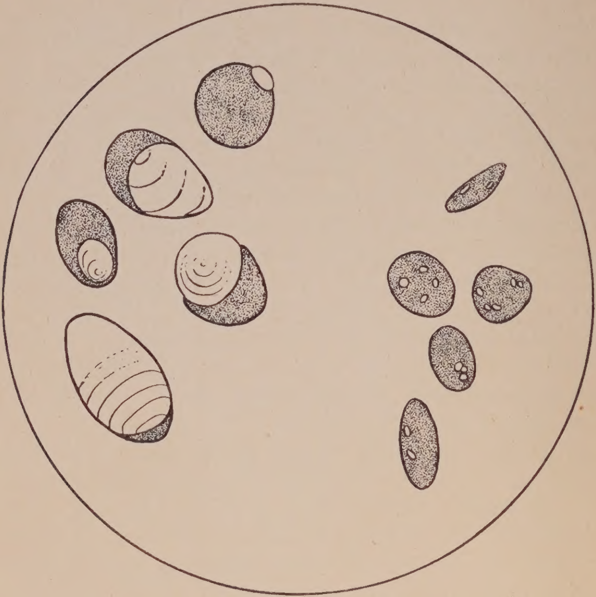


FIG. 8. STARCH GRAINS IN CHLOROPLASTS

Left from assimilating cells of *Pellionia Deveauana*, right from potato plant. The chloroplasts are shaded and the starch grains left white. In *Pellionia* the starch is precipitated in a single large grain, but in the potato plant each chloroplast forms several grains, which do not reach so large a size. The latter arrangement is much more frequent than the former. Highly magnified is saturated, it comes off as small bubbles which form at the surface of the plant, or escape from intercellular spaces inside it.

It has been shown that the volume of oxygen which escapes during normal photosynthesis is exactly equal to the volume of carbon dioxide taken in simultaneously. This means that equal numbers of molecules of the two gases are interchanged, and can be represented by the equation

$$6CO_2+6H_2O \rightarrow \ldots \rightarrow C_6H_{12}O_6+6O_2$$

Such an equation is not very illuminating, however, since it only shows the beginning and end of a long chain of reactions involving many substances, some of which, such as chlorophyll and the 'internal factor' (see p. 28), are not represented in it. The products of photosynthesis and their significance are discussed more fully in the next chapter.

The Energy Transformed in Photosynthesis. Vast quantities of sunlight are transformed every year by green plants. For every gramme of glucose which is synthesized the equivalent of 3,760 calories is taken up. It has been found that assimilating leaves increase their calorific value by about 4,500 for every increase of one gramme in their weight. This is due to the fact that the assimilation process does not stop short at glucose, but goes on to the formation of substances with still higher energy contents. The following table shows the *heat of combustion*, or the energy liberated as heat, when certain substances are completely oxidized to carbon dioxide and water.

Glucose	Carbohydrates	3,760	calories	per gramme.
Sucrose		3,990	,,	,,
Starch		4,100	,,	,,
Cellulose		4,200	,,	,,
Leucine—a protein		6,500	,,	,,
Olive Oil—a fat		9,510	,,	,,

There is no carbohydrate with a heat of combustion sufficiently high to account for the high calorific value found in leaves, and since fats do not occur to any great extent, it would appear that proteins must be formed while assimilation is going on. This question is considered more fully in Chapter III.

The amount of energy fixed in sugar and other molecules is by no means all that the plant absorbs; in most cases, in fact, it is only a very small fraction of the total amount of energy absorbed. The remainder is converted into heat and either causes the evaporation of leaf water, which is called *transpiration*, or is radiated direct into the surrounding air. The following table represents in round figures the partition of light energy falling upon the leaf of a land plant, but it must be remembered that the numbers vary within fairly wide limits according to circumstances.

Incident energy (light)	100
Light fixed by photosynthesis	1
Heat absorbed by transpiration	49
Heat radiated into atmosphere	20
Light reflected or passing through leaf	30

In the unicellular green algae there is no transpiration and less colourless matter to absorb light and transform it into heat, and it was accordingly found in *Chlorella*, a member of this class, that a much greater proportion of the incident light was fixed by photosynthesis than in the case of aerial leaves.

CHEMOSYNTHESIS

Green plants and the organisms, such as animals, fungi, and bacteria, that depend upon them all derive their energy directly or indirectly from sunlight. There are however a few bacteria that live and grow quite independently of light and of the compounds formed by green plants under its influence. They resemble photosynthesizing plants in the sources of their organic material, which are still carbon dioxide and water. These are again built first into sugars, but the necessary energy is not derived from sunlight, as these organisms are able to assimilate in complete darkness. The energy which they absorb is the product of chemical reactions, and various oxidizable substances which are present in soils or pools are made to release energy by oxidation, which causes the formation of sugars in the bacteria. Common examples are sulphuretted hydrogen oxidized by *Beggiatoa*, ferrous salts oxidized by *Leptothrix*, hydrogen oxidized by *Hydrogenomonas*, ammonia oxidized by *Nitrosomonas*, and nitrites oxidized by *Nitrobacter*.

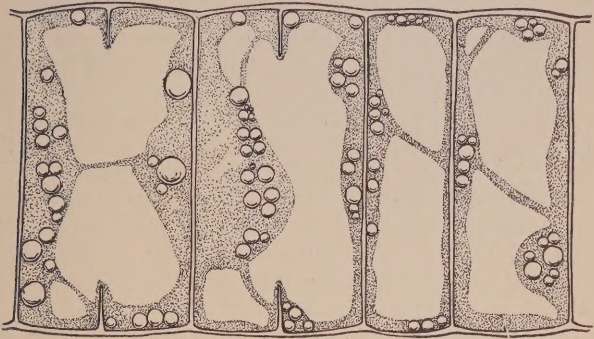


FIG. 9. Cells of *Beggiatoa mirabilis* showing sulphur grains in the protoplasm. (Redrawn after Hinze) × 750

The Sulphur Bacterium. The *Beggiatoa* group of bacteria is found in sulphur springs or bogs, and can exist in the mud upon decaying organic matter. If carbon dioxide and sulphuretted hydrogen, from putrefying material, are present, it becomes independent of the organic matter and carries out its own syntheses. The sulphuretted hydrogen is oxidized to sulphur and water;

$$2H_2S+O_2 \rightarrow 2H_2O+S_2$$

particles of solid sulphur (fig. 9) are formed inside the bacterium and may eventually be further oxidized into sulphates:

$$S_2+2H_2O+3O_2 \rightarrow 2H_2SO_4$$

Free sulphuric acid does not accumulate, but reacts immediately with carbonates entering from the external water:

$$H_2SO_4+CaCO_3 \rightarrow CaSO_4+CO_2+H_2O$$

Energy is released in the oxidations represented in the first two equations and goes to build up sugars from dissolved carbon dioxide. When *Beggiatoa* is kept in tap-water it dies if deprived of either carbon dioxide or sulphuretted hydrogen.

Iron Bacteria. *Leptothrix* and similar organisms possess the power of oxidizing ferrous compounds into ferric hydrate. They occur in ditches and other swampy places, and their presence becomes apparent owing to the rusty-red deposit of ferric hydrate, which may be very considerable. A large proportion of the world's iron deposits are thought to have originated in this way.

Nitrifying Bacteria. A very important class of chemosynthetic bacteria are those which oxidize reduced nitrogenous compounds. Ammonia is produced as a final product of putrefaction and combines with carbon dioxide in the soil to form ammonium carbonate. The oxidation of the carbonate provides the bacterium *Nitrosomonas* with the energy necessary for sugar formation.

$$(NH_4)_2CO_3+3O_2 \rightarrow 2HNO_2+CO_2+3H_2O+148 \text{ calories}$$

The nitrites which are formed by the nitrous acid are further oxidized to nitrates by a second bacterium, *Nitrobacter*, which is thus able to synthesize organic compounds in its turn.

$$Ca(NO_2)_2+O_2 \rightarrow Ca(NO_3)_2+22 \text{ calories}$$

Nitrosomonas and *Nitrobacter* are plentiful in all fertile soils, and the oxidations from which they derive their synthesizing energy have important effects upon the nitrogen contents of such soils. This aspect of their activity is dealt with in the third chapter.

EXPERIMENTAL WORK

The simpler experiments are printed in large type. The value of experimental work is always increased if careful notes, and whenever possible diagrams, are made.

Exp. 1. Reducing Sugars in a Leaf Extract

Cut up a lily, turnip, or other leaf which has been exposed to bright light, and crush it well with a little water. Filter the liquid into a test-tube, which should be about one-third filled. Add about 3 c.c. of Fehling's solution (p. 255) and then boil for a minute.

Fehling's solution is strongly alkaline and contains cupric hydrate, which is deep blue in colour. Substances such as reducing sugars (glucose, fructose, &c.) can reduce the copper in an alkaline medium to cuprous oxide, which is bright red and insoluble. If a reducing sugar was present in your leaf, the blue colour of the Fehling's will disappear on boiling and be replaced by a bright red or brick-red precipitate.

Exp. 2. Reducing Sugars in a Leaf Section

Fill a watch-glass with Fehling's solution, and then cut some transverse sections of a fresh onion leaf. The razor and sections should be moistened during the cutting with alcohol. The sections should be just thin enough to be translucent when mounted. Transfer several sections to the Fehling's solution, place the watch-glass on a water-bath, and allow it to heat slowly. The solution must not be allowed to dry up, and as the volume in the watch-glass shrinks distilled water should be added a few drops at a time from a pipette. While the heating is going on, mount a freshly cut section in water, or dilute glycerine, and keep for comparison. When the watch-glass has been on the water-bath for 5–10 minutes, remove a section with a brush, mount in the same way, and examine under a microscope. The precipitate of cuprous oxide will be visible as minute spherical particles, which appear shiny or black, and not red, owing to their minute size. If some sugar has escaped from the sections, the colour of the precipitate may become visible to the naked eye in the external solution.

Exp. 3. The Presence of Non-reducing and Combined Sugars in Leaves

Take one or two leaves of one of the species named below, and grind them up thoroughly with about 200 c.c. of water in a mortar. Filter some of the liquid into a flask and bring to the boil. Add Fehling's solution, a few drops at a time until there is no further precipitation, and the blue colour remains. Allow the precipitate to settle and filter off the bulk of the clear liquid. Boil this with a few drops of strong sulphuric acid for several minutes. Then make neutral to litmus by adding a few drops of caustic soda. Add about 10 c.c. of Fehling's solution and boil again. A red precipitate will be formed once more, due to the formation of reducing sugars by the hydrolysis, through the agency of the sulphuric acid, of sucrose, a non-reducing sugar, and of various glucosides, i.e. sugar compounds.

Suitable plants are garden nasturtium (*Tropaeolum*) and beet. In the winter greenstuffs, cabbage, brussels sprouts, etc. will be found useful.

Exp. 4. The Formation of Starch from Sugars

Sterilize two beakers by washing them with 1 per cent. formalin, and rinsing out the last traces of the fluid first with freshly boiled water and finally with the experimental liquids. These are a 3 per cent. glucose solution and pure distilled water, both previously sterilized by boiling. Take two destarched *Pelargonium* leaves (from a plant kept for two days in the dark), cut a new surface to the stalk close to the lamina and float one on each liquid. Cover each beaker with a loose piece of glass, and put aside in a dark cupboard for a week. It is necessary during this time that the leaves shall receive plenty of air, so a tight joint between the beakers and their covers must not be made; a cover is necessary to prevent bacterial and fungal spores from entering the solutions. At the end of a week mark the leaf which has been floating on glucose by cutting a notch in it. Dip both leaves into a beaker of boiling water. After 1 minute transfer them to a second beaker containing 70 per cent. alcohol, and allow to stand until the colour is completely extracted. This is greatly hastened by placing the beaker in a water-bath which has been brought to the boil and the flame then extinguished. The beaker should hang into the water, being supported at the rim by the rings of the bath. When the leaves are colourless they will be hard and brittle. Place them on a white saucer and moisten them with tap-water until they are limp again, and then pour off the water. Immerse the leaves in a solution of iodine in potassium iodide, and when there are no further colour changes wash away the iodine with rinsings of water. The leaf which has been floating on glucose will show a dark coloration (due to the presence of starch) especially near the veins along which the glucose has travelled. The leaf which has floated on water will show no coloration. Notice that no light is necessary for this reaction.

Exp. 5. The Uptake of Carbon Dioxide during Starch Formation

Destarch a pot plant of *Pelargonium* by keeping it for two days in the dark. When it is brought back to the light, enclose one leaf in a conical flask (fig. 10 *a*) of suitable size containing about 50 c.c. of a strong solution of caustic soda (*b*). Roll the lamina carefully to pass it through the neck of the flask. Wrap a piece of cotton-wool, soaked in lime-water (*c*), round the petiole, and close the flask with a holed and split rubber cork (*d*). This will make a joint sufficiently impermeable to carbon dioxide. If an ordinary cork has to be used, its surface should finally be smeared with vaseline. Support the flask by means of a retort stand, taking care not to crack the petiole or let the leaf touch the caustic soda. Place the whole in direct sunlight or about 9 in. from a 50 c.p. electric lamp for not less than four hours, and then test the enclosed leaf for starch as in experiment 4. Test an untreated leaf similarly. Both leaves should be young (near the apex of the plant), as old leaves are much slower in forming starch. The enclosed leaf will contain no starch as no carbon dioxide was available.

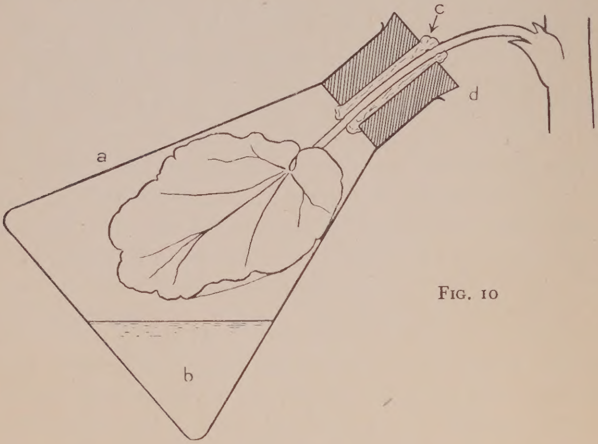


FIG. 10

Exp. 6. THE CONNEXION BETWEEN STOMATA AND AIR SPACES OF THE LEAF

Cut an onion leaf low down, and push the end of a piece of glass tubing into the cavity. Bind the leaf on firmly with wool or soft cotton. Immerse the leaf in water, and blow gently into the tube. Bubbles of air will be forced out of the stomata.

Rub one side of the leaf with a moistened finger, until the waxy bloom is removed. On further blowing, bubbles will no longer come out on the rubbed side. This is because its surface is now thoroughly wetted and the stomatal pores blocked by water.

If onion leaves are not available, other fistular organs, such as the flower stalks of *Narcissus*, may be used.

Exp. 7. AIR-SPACE SYSTEM OF THE LEAF

Fit a new rubber cork (fig. 11 A) having one hole, with a glass tube (B) drawn into a jet at the lower end. The cork is to be fitted into a small filter-flask (C) so that the tube reaches almost to the bottom. Fill the flask with tap-water almost up to the side tube, which is to be attached to a filter-pump by a long piece of pressure tubing. To the upper end of the tube (B) attach a piece of new pressure tubing (D) about 2 inches long. Unless the rubber used is new and springy, there will be difficulty in obtaining the necessary air-tight joints. Select a twig of cherry laurel (*Prunus laurocerasus*) and cut off a terminal piece with four or five leaves attached. Make sure that the surfaces of leaves and stem are unbroken. The stem of the piece selected must be straight, without leaves for the lowest inch, and about 4 mm. diameter at the cut. The sprig must not be trimmed or cut in any way. Smear the sides of the straight stem above the cut with a little vaseline (avoid the cut surface), when it will be found easy to push it well into the upper end of the pressure tubing (D) without damaging the bark. Stand the entire apparatus in a large inverted bell-jar and connect with the filter-pump. Run water into the jar until the upper end of the pressure tubing

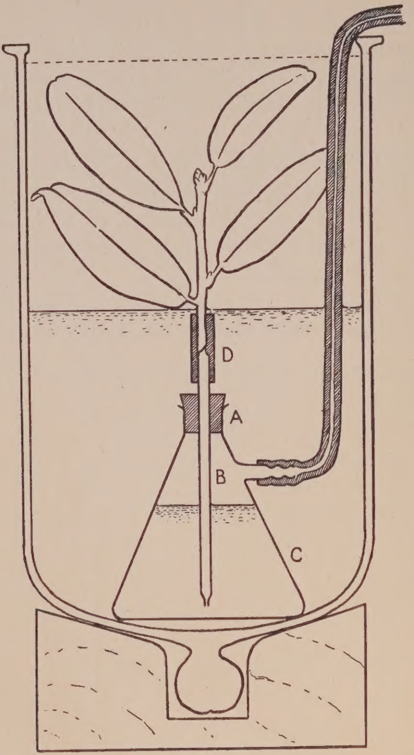


Fig. 11

is just immersed, then turn on the pump. Bubbles will at once begin to escape from the jet, showing that air is being drawn through the leaves and stem into the flask. Adjust the rate of bubbling by altering the rate of flow through the pump until the bubbles can easily be counted. Take several half-minute readings of the rate, which should remain fairly constant.

Pour further water into the bell-jar until the shoot itself is completely immersed. The rate of bubbling will greatly decrease, since air can no longer enter the stomata. It will not stop, because air in the internal air spaces is slowly drawn out. After about half an hour, dark patches will be noticed on the under surfaces of the leaves, showing that water has entered through the stomata and is filling the spaces previously occupied by air.

Exp. 8. Carbon Dioxide enters the Leaf through the Stomata

Cut two leaves from a *Pelargonium* plant which has been destarched by keeping it for two days in the dark. These leaves have stomata on their lower surfaces only. Vaseline the under side of one leaf and the upper side of the other, and place both in a bright light with their petioles dipping into water. Leave for at least four hours and then wipe off with a soft rag as much vaseline as possible without damaging the leaf. Mark the leaf vaselined on top by cutting a notch in its lamina, and test both for starch by the iodine method.

The notched leaf will show a blue colour due to the starch which has formed from the carbon dioxide absorbed. The other, in which the stomata were blocked, will show none.

Exp. 9. The Closure of Stomata due to Plasmolysis of the Guard Cells

Strip a piece of epidermis from a lily, iris, or rhubarb leaf, and divide it into two pieces. Put one piece into a watch-glass containing a strong solution (about 33 per cent.) of calcium chloride, and the other into a watch-glass of distilled water. After 5–10 minutes, transfer the pieces to a drop of the same medium on microscope slides, cover with coverslips, and examine. The stomata in the calcium chloride solution will be closed, whereas those in distilled water will be open.

Exp. 10. THE FIXATION OF LIGHT

Make an envelope of dull black paper just large enough to slip over and enclose the lamina of any selected leaf; it should be more than three inches square. Cut a simple design in one face of the envelope, and then fix it on to a destarched leaf with the stencil uppermost. Close the envelope with paper clips across its open end, and place further clips to keep the paper in close contact with the leaf, especially round the cut edges. No light must be allowed to get between the leaf and the paper.

The experiment is best performed with a young leaf attached to a plant rooted out of doors, but a pot plant will do. Destarching may be carried out beforehand by enclosing the leaf in a black paper envelope with no stencil cut in it. Potato leaves require 12 to 24 hours' darkening followed by about 4 hours' exposure to light; *Pelargonium* leaves 48 hours and 4 hours respectively. At the end of the exposure cut off the leaf, and test for starch as described in experiment 4. It will be found that a dark-blue pattern, representing the cut-out part of the envelope, is formed on the colourless background of the leaf. Starch formation is thus seen to be limited to the region which received light. The formation of starch is so sharply limited, that if a photographic negative is placed over a destarched leaf, a good print may be 'developed' on the leaf, after exposure to light, by the iodine treatment.

Exp. 11. THE NECESSITY FOR CHLOROPHYLL

Cut a variegated leaf of *Pelargonium* of a 'bicolor' variety from a plant that has been exposed for several hours to bright light. Soak the leaf thoroughly in tap-water, and place it on a dark background under a flat piece of glass. Lay transparent paper on the glass, and trace the outline of the leaf, and the divisions between the green and colourless portions. Decolorize the leaf and stain with iodine as in experiment 4. Finish by soaking the leaf in water until the outline again coincides with that originally traced on the paper. Compare the area stained blue-black with the area you have drawn as originally green. Notice that starch is only formed where chlorophyll is developed.

Exp. 12. EXTRACTION OF CHLOROPHYLL

Chop up a handful of grass cuttings, and put into a flask with about 100 c.c. of 85 per cent. acetone. Shake round and allow to stand until the colour has passed into the acetone. The extraction can be made more rapid by placing the leaf material in a filter-funnel, and drawing the acetone through several times. When the acetone has become a deep green, view the solution by reflected light. A characteristic blood-red fluorescence is visible.

Pour some of the extract into an evaporating basin, put aside and allow to dry up. Minute crystals of the chlorophyll pigments will be formed.

Drop a spot of the solution on to a clean piece of filter-paper. As it spreads itself, notice that the yellow pigments travel fastest, forming a narrow yellow circle round a green centre.

Exp. 13. THE CHLOROPLAST THE SITE OF PHOTOSYNTHESIS

a. Mount a moss leaf in a solution of chloral hydrate, and examine under the microscope. Notice that the green colour is confined in little granules, the chloroplasts. Put a second leaf into a watch-glass full of Schimper's solution. After 2–3 minutes transfer the leaf to a drop of the reagent on a slide, cover, and examine under the microscope. The iodine in the Schimper's solution will have stained the starch present in the leaf. Notice that it is confined to the chloroplasts.

b. Cut a transverse section of a leaf in a piece of split pith, and treat as above. Notice the distribution of the chloroplasts and of the starch. Starch formation only goes on in the green plastids. Suitable leaves are those of cherry laurel and St. John's wort (*Hypericum calycinum*).

Exp. 14. The Formation of Free Oxygen

Put enough water-weed into a 500 c.c. flask to fill the bulb loosely. Enrich some tap-water with carbon dioxide. This may conveniently be done by bubbling carbon dioxide from a Kipp into a large flask about half filled with the water. When bubbling has gone on for about five minutes stopper the flask and shake. Transfer this solution of carbon dioxide to the experimental flask, which must be completely filled. Invert the flask, and place with the open end dipping into water in a trough. This need not necessarily contain extra carbon dioxide. Support the flask by means of a ring or clamp, and place the whole in a bright light.

Notice that gas-bubbles immediately begin to escape from the plants, especially at the broken ends of the stalks, and collect round the surface of the flask. When a sufficient quantity of the gas has been collected, stopper the flask under water, and place with the neck upwards to collect the gas. Bubbles which stick to the glass surface may be detached by rotating the flask rapidly while holding the bulb in one hand. There should be about an inch of gas down the neck of the flask. Uncork and put in a glowing splint to recognize the gas, which proves to be oxygen.

Suitable plants for this experiment are Starwort (*Callitriche*), Water Buttercup (*Ranunculus aquatilis*), Canadian Pond Weed (*Elodea canadensis*), or Pondweeds (species of *Potamogeton*).

II

SUGARS AND THE PLANT MATERIALS WHICH ARE FORMED FROM THEM

THE CHEMISTRY OF THE CARBOHYDRATES

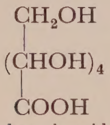
A GREAT many substances containing carbon, hydrogen, and oxygen in their molecules occur in plants and are derived either directly or indirectly from the sugar formed by photosynthesis. In the first part of this chapter their most important chemical characters are described, and in the latter part their behaviour in plants.

The most important of these substances belong to the class called *carbohydrates*, which includes the sugars themselves and the substances which are formed by the union of two or more sugar molecules. The common carbohydrates can be arranged in the following scheme:

Monosaccharides:	
Pentoses, e.g. arabinose.	Soluble in water.
Hexoses, e.g. glucose, mannose, fructose.	
Disaccharides, e.g. sucrose, maltose.	
Polysaccharides, e.g. starch, cellulose.	Insoluble.

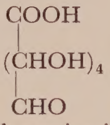
Monosaccharides. The *pentoses* differ from the hexoses in possessing five carbon atoms linked together in a chain, whereas the hexoses have a chain of six. The general formula for all pentoses is $C_5H_{10}O_5$, and for all hexoses, $C_6H_{12}O_6$. Pentose sugars differ from one another in the way in which their hydrogen and oxygen atoms are grouped round the carbon chain. Like all other monosaccharides and disaccharides, they are readily soluble in water, and in this respect they differ sharply from the polysaccharides. Pentoses are rarely found in plants except in very small quantities, but they give rise to polysaccharides and other compounds, which are the gums and mucilages of succulent plants, seaweeds and others.

Hexoses. Hexoses are much commoner and much more important in plants, and include a large number of different substances, all of which are readily soluble and more or less sweet to the taste. In the majority of their chemical properties they resemble one another closely, but differ among themselves in certain important respects. They are all fairly easily oxidized into organic acids, and by complete oxidation are converted to carbon dioxide and water. Glucose, for example, passes with increasing degrees of oxidation into gluconic, glucuronic, and saccharic acids.



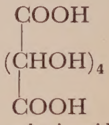
$$\begin{array}{c}\mathrm{CH_2OH} \\ | \\ \mathrm{(CHOH)_4} \\ | \\ \mathrm{COOH}\end{array}$$

gluconic acid



$$\begin{array}{c}\mathrm{COOH} \\ | \\ \mathrm{(CHOH)_4} \\ | \\ \mathrm{CHO}\end{array}$$

glucuronic acid



$$\begin{array}{c}\mathrm{COOH} \\ | \\ \mathrm{(CHOH)_4} \\ | \\ \mathrm{COOH}\end{array}$$

saccharic acid

The complete oxidation can be represented as

$$\mathrm{C_6H_{12}O_6+6O_2 \rightarrow 6CO_2+6H_2O}$$

but the reaction no doubt takes place in a series of stages, which cannot be stated at present.

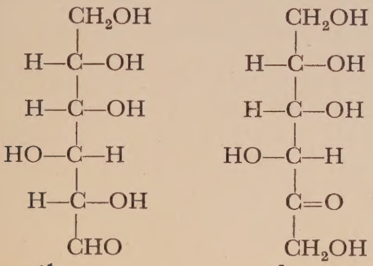
The reducing power of the hexoses is shared by other monosaccharides and even by some disaccharides, such as maltose. Maltose can reduce because one of the two glucose molecules which condense into the maltose molecule retains its reducing properties unimpaired. In alkaline solutions these sugars will reduce cupric hydrate to cuprous oxide:

$$\mathrm{2Cu(OH)_2—O \rightarrow Cu_2O+2H_2O}$$

Cuprous oxide is an insoluble red solid, and this reaction can be used to detect the presence of reducing sugars and to estimate their concentration in simple solutions. It is carried out in a large number of modifications, but the one most frequently used is known as Fehling's reaction, and is described in detail at the end of the previous chapter (p. 35).

Glucose is called an aldehyde hexose because it possesses the group $—C\begin{smallmatrix}\diagup H\\ \diagdown O\end{smallmatrix}$. Fructose does not possess this group, but owes its reducing power to the presence of a ketone group $>C=O$ in its molecule. In other respects glucose and fructose are very similar and are readily converted from one to another in the presence of strong alkalis. At the same time another aldohexose, called *mannose*, appears, and also numerous other sugars in small quantities. It is interesting to notice that those which are formed in the greatest quantity, and which may therefore be regarded as the most stable, are all hexoses. This molecular stability is probably the reason why hexose sugars and their derivatives are so much commoner than others in plants.

The structural formulae of glucose and fructose are given below, and it will be seen that in the greater part of their molecules they are identical.



$$\begin{array}{c}CH_2OH\\ |\\ H—C—OH\\ |\\ H—C—OH\\ |\\ HO—C—H\\ |\\ H—C—OH\\ |\\ CHO\end{array}\qquad\qquad\begin{array}{c}CH_2OH\\ |\\ H—C—OH\\ |\\ H—C—OH\\ |\\ HO—C—H\\ |\\ C=O\\ |\\ CH_2OH\end{array}$$

glucose **fructose**

Substances such as glucose and fructose which possess the same atoms in the same numbers, but differ in the manner of their grouping, are called *structural isomers.* It is impossible to understand the sugars, without also knowing another kind of isomerism, called *stereoisomerism*, in which both isomers have the same groupings, and differ only in the arrangement of them in space. Aldehyde glucose, for example, exists in two forms, and is actually two substances, not one. It is not possible to represent the differences between them 'on the flat' because they are brought about by differences of distribution 'in the solid'. They are, however, always of such a kind that one isomer is the mirror image of the other, and no rearrangement of the molecules as a whole will make them coincide (fig. 12). Substances which exist as stereoisomers always have the property of

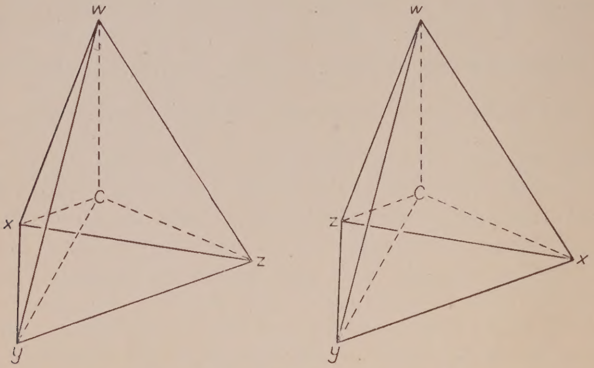


FIG. 12. THE SPACE ARRANGEMENT OF CARBON ATOMS AND ASSOCIATED GROUPINGS

Each carbon atom (*C*) is situated at the centre of a tetrahedron, and the linkages, shown by broken lines, run to the groups *w*, *x*, *y*, *z*, which are combined with it at the four corners of the figure. There are two possible arrangements of *w*, *x*, *y*, and *z* about the carbon atom, such that the two tetrahedra (molecules) do not coincide, however they are reorientated. There are in consequence two separate substances to correspond rotating the plane of polarized light.[1] If one isomer causes dextro-, or clockwise, rotation, the second isomer always causes laevo-, or counter-clockwise, rotation. There is thus a d-glucose (dextro glucose) with dextro-rotatory powers, and a corresponding l-glucose, with laevo-rotatory powers. When glucose is formed artificially from fructose, dextro- and laevo-forms appear together, but in the plant l-glucose is apparently never formed, all the sugar appearing in the dextro-shape.

Disaccharides. Glucose and fructose are very active substances in solution. They undergo still further rearrangements within their molecules when dissolved, and are able to interact with a large number of substances. A molecule of glucose may also condense with a molecule of fructose, when the disaccharide, *sucrose*, or cane sugar, is formed.

$$\underset{\textbf{glucose}}{C_6H_{12}O_6}+\underset{\textbf{fructose}}{C_6H_{12}O_6} \rightarrow \underset{\textbf{sucrose}}{\overset{12}{C_{\cancel{6}}}H_{22}O_{11}}+H_2O$$

In plants this reaction goes on very readily indeed, owing to the presence of a suitable catalyst, but sucrose itself cannot be condensed into any more complex form, and is in fact a much less active substance altogether. It is very common in plants and is often found in considerable quantities in storage organs, the stem of sugar-cane and the root of sugar-beet being outstanding examples. It is always found, in addition, in healthy leaves, and few plant organs are entirely without it. It is readily hydrolysed back to glucose and fructose by boiling in the presence of a little acid.

---

[1] In a narrow beam the rays of light are supposed to be vibrating in all planes. Certain prisms have the property of stopping all the rays except those vibrating in one plane only, and the light which passes through them is said to be polarized. If such light is passed through a sugar or other similar solution, the plane of the vibration is rotated, as is shown by the fact that a second prism placed in its path after leaving the solution must be rotated from the original plane before it will allow the light to pass through.

Maltose, also a disaccharide, is sometimes found. It has the same empirical formula as sucrose, but is formed by the condensation of two molecules of glucose, fructose taking no part in its formation.

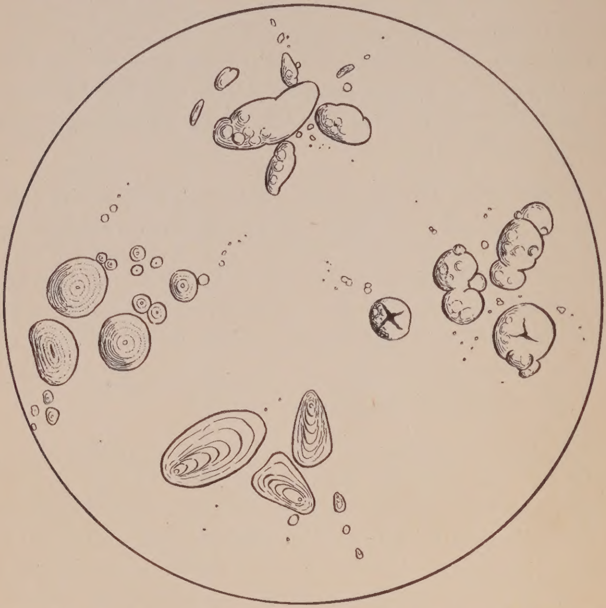


FIG. 13. STARCH GRAINS ISOLATED FROM THE CELLS OF RESERVE TISSUES OF VARIOUS SPECIES

Top from cotyledons of broad bean; left from endosperm of barley; bottom from tuber of potato; right from cotyledons of pea

Polysaccharides. These differ from mono- and disaccharides by being insoluble in water, or soluble only to a slight extent. They are derived from monosaccharides in a similar way to disaccharides, that is to say, by condensation of the simple molecules. A single polysaccharide molecule contains many sugar molecules, the exact number not yet being known for any single case, though it may run into hundreds. The commonest polysaccharides in plants are *starch* and *cellulose*. The former appears as granules inside living cells, the shape of the grains varying in different species, and being to some extent characteristic (fig. 13). It is very easily recognized by the blue colour which it gives with iodine in the presence of water. In the absence of the latter, if for example an alcoholic solution of iodine is used with dry material, there is no reaction. Starch has not yet been synthesized artificially though it is formed very plentifully by the great majority of plants. It can be hydrolysed by boiling for some time with dilute acid. If the hydrolysis is carried out cautiously, a number of stages can be recognized, some of them by the colours given with iodine; they are usually described as follows:

Substance	*Iodine colour*
Starch	Blue
Soluble Starch	Blue
Erythrodextrin	Purple to red
Achrodextrin	None
Maltose	None
Glucose	None

Hydrolysis ↓

Condensation ↑

The complexity of the starch molecule may be judged from the fact that soluble starch has an empirical formula approximating to $C_{1200}H_{2000}O_{1000}$. Each substance in the hydrolysis series is simpler than the one above it; the dextrins for example are still polysaccharides, but have simpler molecules than starch. Maltose is a disaccharide which can be further hydrolysed and then yields only glucose. The starch molecule must, therefore, consist of a large number of glucose molecules condensed together without the presence of any other sugar, so that we can have no doubt as to the sugar from which starch has to be formed in plants. Starch molecules are not all identical; in other words there is not one starch but many, depending for their natures upon the different numbers and different ways in which their glucose molecules are joined together.

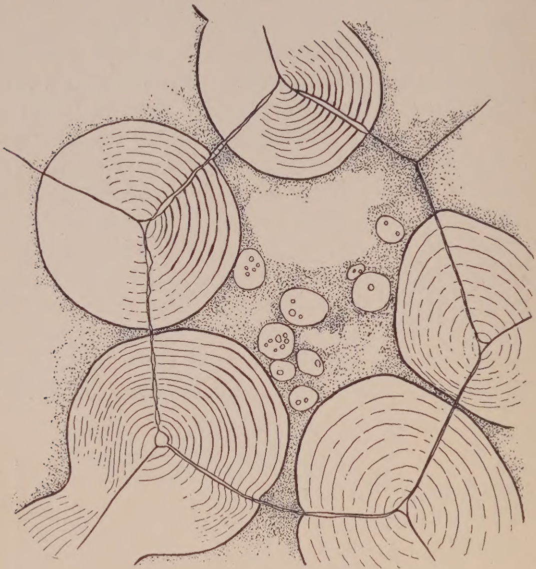


FIG. 14. CELL FROM THE COTYLEDONS (STORAGE TISSUE) OF *Lupinus pilosus*

The abundant cellulose is deposited in layers upon the originally thin cell wall. Within the protoplasm are further reserves of oil and protein. Highly magnified

Cellulose is very similar to starch in its composition. Its molecule is even larger, but on complete hydrolysis glucose alone is obtained. Maltose is not formed, however, but a similar disaccharide called *cellose*, which has not yet been found in plants in the free state. A reason for this is suggested by the law of linked reactions (*see* p. 62). Cellulose is inert and very insoluble in most reagents. It is deposited round the outside of living protoplasts and forms the cell walls, and eventually a very large proportion of the plant. Notable examples of pure cellulose walls are afforded by the hairs of the cotton plant (*Gossypium*) and by the storage tissue of lupin seeds (fig. 14). In older cell walls the cellulose often becomes impregnated or combined with various complex materials, whose chemistry is little known; *lignin* and *cutin* are substances of this kind. Pure cellulose which, like starch, is really a number of closely related compounds, on being treated with sulphuric acid gives *amyloid*, the only substance besides starch which will give a blue reaction with iodine (*see* **exp. 19**).

Numerous other polysaccharides are formed in plants, but are usually restricted to certain families or other large groups, whereas starch and cellulose are almost universal. *Glycogen*, also a condensation product of glucose, is found in the fungi, where it is said to replace starch. *Inulin* is derived from fructose, and is only formed in members of the family *Compositae*. Other sugars such as mannose, galactose, and the pentoses also give rise to polysaccharides in plants, sometimes more than one sugar being involved, as is usually the case with the mucilages.

Glucosides. The combining powers of the sugars are not limited to other molecules of a similar kind, and glucose especially is found combined with an immense variety of other substances in plants. Any compound in which a sugar molecule is concerned is called a glucoside, whatever the sugar may happen to be. Some of the best-known classes of glucosides are mentioned in the following list, together with the substances they yield on hydrolysis.

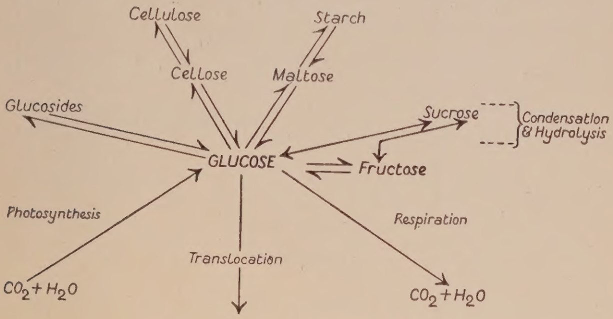
Glucosides	*Products of hydrolysis*	*Occurrence*
Anthocyanin pigments	Glucose and anthocyanidins	Purple, blue, and red flowers
Flavone pigments	Various sugars and flavones	Cream coloured flowers, etc.
Cyanogenetic glucosides	Glucose, benzaldehyde and prussic acid	Bitter almonds and laurel leaves
Mustard oil glucosides	Glucose, mustard oil, and $KHSO_4$	Cruciferous plants

The anthocyanidins are complex organic substances and their glucosides give purple, blue, and red colours to the cell sap in which they are dissolved. They form the pigments of many flowers. The flavones are of similar structure, and may also give rise to flower colours, in this case creams and yellows, though their glucosides are colourless.

It should be noted that although a large number of carbohydrates are formed in plants, the great majority of them belong to the hexose series, and that among the hexoses, glucose and its derivatives play the most prominent part. This is true of plants as a whole; there are, however, certain groups, such as the cacti, in which the pentoses are the most important.

THE CARBOHYDRATES OF THE LEAF

It is convenient to regard photosynthesis as ending with formation of a hexose sugar, such as glucose, and this is done, for example, in the 'assimilation equation' given on p. 31. There is no real break at this point, however, and the sugar as soon as it is formed begins to undergo further changes. Glucose is a very reactive substance which can enter into a large variety of chemical changes, and its behaviour in solutions outside the plant has already been described (p. 46). Inside the plant conditions are more complex, because catalysts are present, which bring about the formation of substances that do not arise in a simple solution. These are very numerous and are derived from the photosynthesized glucose in a number of different ways. Some of them, such as starch and sucrose, are formed in the chloroplasts themselves, though light is not required for their formation. Glucose is continually passing away from the chloroplasts into the surrounding tissues, however, and may eventually be changed into other substances at a considerable distance from the chloroplasts in which it originated. The cellulose walls formed at the growing points of the stem and root and the starch laid down in storage organs are examples. The following diagram illustrates the principal relationships of the glucose formed in the leaf.


Cellulose
Starch
Cellose
Maltose
Glucosides
Sucrose
Condensation & Hydrolysis
GLUCOSE
Fructose
Photosynthesis
Respiration
Translocation
$CO_2 + H_2O$
$CO_2 + H_2O$

The Leaf Sugars.- Whenever a fresh leaf is examined it is always found to contain sugars, whether photosynthesis has been going on recently or not. Sucrose, glucose, and fructose are rarely absent, if ever, but the amount present varies considerably, especially in the case of sucrose. The time of day, season, and external conditions all exercise marked effects on these concentrations. The sugar content of mangold leaves at different times of day has been very carefully investigated and illustrates what happens in the leaf during periods of light and darkness. This leaf only forms starch when very young, and by examining adult leaves it was possible to rule out the complications introduced by its presence. Fig. 15 shows how the different sugars behaved during a day in summer, when photosynthesis went on rapidly.

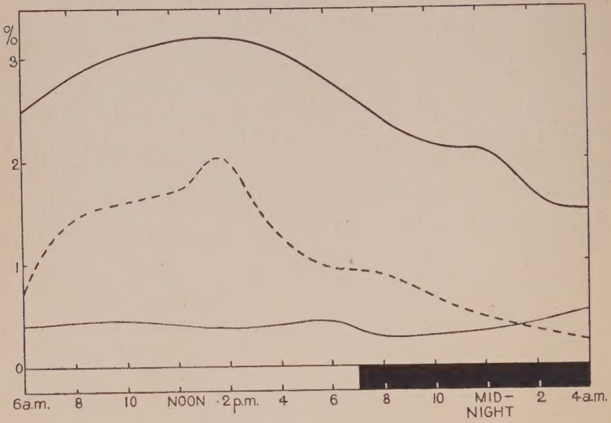


FIG. 15. Graph of the amount of sugar in the leaf of mangold (*Beta vulgaris var.*) throughout a day and night in August. Heavy continuous line = sucrose; broken line = hexose; thin line = pentose. Period of 'complete darkness' shown in black at the bottom. (From the data of Davis, Daish and Sawyer)

It is very difficult to determine quantities of glucose and fructose separately when both are present in a complex solution, and the curve for hexoses in the figure includes both these sugars. The amounts of hexoses and sucrose, the only sugars present in any quantity, increase rapidly in the leaf for the first few hours after dawn, but come to a stop in the middle of the day, when the light is still bright. This is caused by the fact that the stomata regularly close at this time, preventing the diffusion of further carbon dioxide into the leaf. This early closing of the stomata occurs also in sugar-beet and a good many other plants, especially the cereals. Once the closure has taken place, the hexose and sucrose contents of the leaf decline, as sugar is slowly removed by translocation.

The Relation between Sucrose and Hexose. It has been suggested that sucrose is formed first in photosynthesis and that the glucose and fructose found in leaves are derived from it by hydrolysis. The suggestion rests principally upon the fact that when photosynthesis is going on more sucrose is formed than hexose. It will be seen by referring to fig. 16 that the same remark applies to starch in starch-forming leaves, but nobody has suggested that starch is formed before glucose. There is, in fact, no basis for the idea that sucrose is the first formed sugar, and although it appears practically simultaneously with the hexoses in leaves, there can be little doubt that it is always formed by the condensation of glucose and fructose, as would be anticipated from their general chemistry (p. 46 et seq.).

The quantitative relation between sucrose and hexose in these leaves is dependent also upon the season. As the summer advances the amount of sucrose to be found in the leaves gradually increases, but the amount of hexose increases much more rapidly until in the end it is actually more plentiful than sucrose. This would seem to imply that hexose is not so easily transformed to sucrose as at first; but the reason for such a change is still a mystery.

The behaviour of these sugars in stems differs considerably from their behaviour in leaves, and has been worked out in great detail in the stems of the sugar-cane. The total amount of sugar increases steadily during the growing period, but the increment is due almost entirely to the heaping up of sucrose. Hexose reaches its maximum concentration at an early period and thereafter slowly declines.

The state of affairs in the swollen root of the sugar-beet is very similar; practically the whole of the sugar which accumulates is in the form of sucrose. The interpretation of these relationships will be clearer when the polysaccharides have been considered.

The Relation between Glucose and Fructose. In alkaline solutions glucose, fructose, or mannose pass readily into one another until finally a mixture of all three sugars is set up. In plants glucose and fructose similarly appear together, but mannose is completely suppressed. The protoplasmic medium is not strongly alkaline, however, and the reactions which go on in it are catalysed instead by substances called *enzymes*, which will be described later. Under enzymic influence glucose and fructose are interconvertible in the plant, as outside, but the reaction is not a very fast one, as is shown by the following experiment. The fungus *Sterigmatocystis* was grown upon a mixture of glucose and fructose. At the start 3·5 gm. of each were present, but when the fungus was caused to respire at an unusually high rate 1 gm. of fructose remained when all the glucose had been used up. Glucose was the sugar affected by respiration, and as it disappeared fructose was slowly converted into glucose to replace it, though at a much slower rate than that at which the glucose was removed.

Starch. In many leaves starch appears in the chloroplasts whenever photosynthesis is sufficiently rapid. It can easily be shown, however, that light is not necessary for its formation provided that a supply of sugar is ensured (**exp. 4**, p. 36). The steps in its formation are believed to be the reverse of those recognized during hydrolysis, the sequence of substances being glucose, maltose, dextrins, soluble starch, and finally starch itself. There is evidence which suggests, however, that we are not dealing with a series of reversible reactions, in the strict chemical sense, so it is possible that the intermediate compounds may be different in the up and down grade series. Both go on under the influence of several enzymes, the collective name for which is *diastase*. Fig. 16 shows the amount of

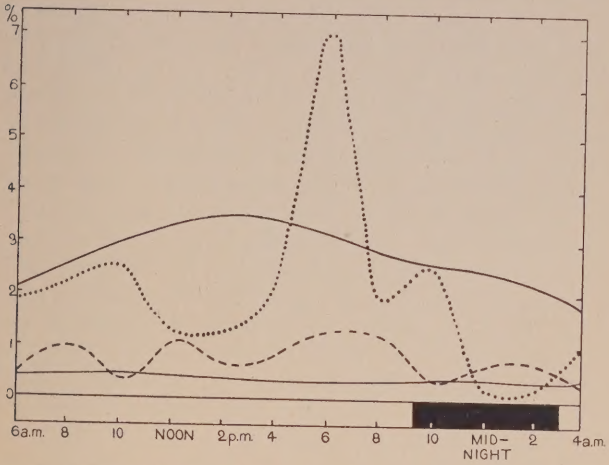


FIG. 16. Graph of the amount of starch and sugar in the leaves of the potato plant throughout a day and night in July. Dotted line = starch; heavy continuous line = sucrose; broken line = hexose; thin line = pentose. Period of 'complete darkness' shown in black at the bottom. (From the data of Davis, Daish and Sawyer)

starch and sugars found in leaves of the potato plant at different times of day.

Starch formation only goes on within a limited range of sugar concentration. Until a certain amount of sugar is accumulated none at all becomes converted to starch, and if the concentration becomes very high starch is no longer synthesized, and that already formed is changed back to sugar. The minimum sugar concentration at which starch is formed is called the *lower critical concentration*, and the point at which synthesis comes to a stop the *upper critical concentration.*

The actual value of the lower critical concentration is very variable; the lowest case on record is that of the moss, *Mnium*, which formed starch when placed in a 0·1 per cent. solution of sucrose. From this low figure all gradations are found up to the onion leaf (*Allium cepa*), which has never been induced to form starch at all. Leaves which have low critical concentrations, and hence form starch very readily, are called 'starch leaves', while those which have high critical concentrations and consequently accumulate a good deal of sugar are called 'sugar leaves'. Most dicotyledons have starch leaves, with a critical concentration of 0·2 to 0·5 per cent. The distinction is by no means a sharp one, however. Critical concentrations may vary not only in different plants, but also in different parts of the same plant. In potato tubers, where starch accumulates very extensively, the lower critical concentration is distinctly below that of the leaves, where starch is also formed. Season exercises a marked effect also, and it is well known that evergreen shrubs, which form starch readily in summer, in the winter accumulate sugar instead. Sucrose is formed in the summer as well as starch, but in the winter more material takes the sucrose turning, and starch eventually disappears. The alteration of critical concentration is said to be associated with the different temperatures at the two seasons, but certain information is lacking.

The upper critical concentration lies in the neighbourhood of 30 per cent., but like the lower concentration is probably very variable. It plays an important part in the physiology of guard cells, which form high concentrations of sugar when illuminated, so that the starch in them disappears to form still more sugar, greatly increasing the osmotic pressure of the cell sap. This takes place in the stomata of both starch and sugar leaves, since even the latter form starch in their guard cells. It has also been observed that leaves which are exposed to very bright light eventually lose the starch which they formed at first; this is also due to the concentration of sugars exceeding the upper limit.

Glucosides. Many substances with which sugars can combine are found in leaves, and it is, therefore, not surprising to learn that many sugar compounds, or glucosides, are formed during photosynthesis as well as the carbohydrates already mentioned. The behaviour in leaves of a few of the many known glucosides has been carefully examined and has been found very similar to that of sucrose, that is to say, the glucosides accumulated while photosynthesis was going on and slowly disappeared in the dark. There is thus no reason to suppose that they differ in their origin and decomposition from the more plentiful sugar derivatives. Some of the substances with which the sugars combine, such as prussic acid, are strong poisons, and it has been suggested that their combination prevents injury to the cells. This may be so, but does not, of course, explain why the glucosides are formed; this is simply due to the chemical natures of the substances involved and their powers of uniting together.

RESERVE SUBSTANCES

Metabolism is a general term for the building up of complex substances inside the organism, and their subsequent degradation during respiration and associated changes. During its course many of the substances which are formed heap up in various parts of the plant, though later they may be reconverted to simpler forms, and so disappear again. Materials which behave in this way are often called *reserves*, since it is assumed that they are only brought into active play when more available substances have been used up. It must not be supposed, however, that this storage is in any way analogous to the 'laying up against a rainy day' practised by human beings. To explain what is going on it is first necessary to describe the nature of certain chemical sequences.

Linked reactions. In plants we rarely have to deal with single isolated reactions, but rather with chains of reactions which are linked together, the end products of one reaction being the starting-point for the next. Thus we may have the series of reactions

$$A \rightarrow B \rightarrow C \rightarrow \ldots\ldots\ldots\ldots\ldots\ldots X$$

in which A is being converted into X, through a series of intermediate substances, B, C, etc. Each reaction in the chain A→B, B→C, and so on, has its own specific rate per unit of reacting material, so that A→B may be faster than B→C, or vice versa. If A→B is the faster reaction, the substance B will be formed more rapidly than it is converted into C, and hence it will tend to accumulate. The actual rate at which B is transformed depends, however, not only on the rate per unit amount of B, called the *velocity constant* of the reaction, but also on the amount of B which is reacting. Hence as B heaps up the rate of its transformation to C is also increased, until finally it becomes equal to the rate of formation of B. Similar adjustments occur all along the series to X, and when they are complete matter is passing at the same rate through every reaction of the sequence, which is then said to be in *dynamic equilibrium*. The behaviour of X depends upon two circumstances; if, for example, the reactions are reversible, as is usually the case, when X has reached a certain concentration the 'back reaction' will become as fast as the formative reaction, and the concentration of X will then remain constant, instead of augmenting further. The case is altered considerably if X happens to be only slightly soluble; the solution then rapidly becomes saturated, and X is precipitated out. The solid material is chemically inactive and the effective concentration is restricted to the small amount in solution, which remains constant, and is never great enough to prevent further formation. Under these conditions X may accumulate in the solid form indefinitely.

If the case of starch is now examined, it will be seen that we are dealing with an insoluble end product, such as X in the foregoing example. Glucose is converted through various intermediates into starch, and at medium concentrations the more glucose there is present the faster the formation of starch goes on; thus when photosynthesis is rapid and much glucose is being formed the synthesis of starch is correspondingly accelerated. If continuous lighting is supplied, starch continues to accumulate in the leaf practically indefinitely. The breakdown of starch to glucose is always going on simultaneously, however, and if photosynthesis stops, and glucose is steadily removed by translocation, starch synthesis is retarded, and finally becomes slower than its decomposition by hydrolysis. Under these conditions the precipitated starch slowly goes back into solution, and disappears from the leaves, being translocated as glucose, or perhaps as other sugars.

It is thus easy to understand that insoluble substances formed during metabolism are very liable to accumulate, and so become 'reserves'. Sucrose, on the other hand, is an example of a soluble substance which readily accumulates. It is the product of the condensation when glucose and fructose come together, and itself has no power to condense into more complex substances. We have thus the system

$$\text{glucose}+\text{fructose} \underset{\text{hydrolysis}}{\overset{\text{condensation}}{\rightleftarrows}} \text{sucrose}$$

and sucrose heaps up until the rate of hydrolysis becomes equal to the rate of condensation. Inside plants the position of equilibrium is not constant, however, but is liable to variations of the kind described on p. 57.

THE FATS

Fats or oils occur in almost all plants, but are especially abundant in the seeds of angiosperms, among which they are rare in the vegetative organs. They form, in fact, the typical reserve substances of seeds (see fig. 69), and starch replaces them only in two families, the *Gramineae* and *Leguminosae*. Nearly all plant fats are liquids at ordinary temperatures, and in common language are called oils in consequence. They are immiscible with water, but on vigorous shaking they form fine emulsions, from which they may take a long time to separate out again. Chemically they are esters formed by the union of glycerine, an alcohol with three –OH groups, and some organic acid. A large number of such acids, which are distinguished by the name of fatty acids, can enter into the formation of fats. The reaction can be written as follows, the group represented by R varying according to the nature of the fatty acid:

$$\mathrm{C_3H_5(OH)_3+3R.COOH \rightarrow C_3H_5(R.COO)_3+3H_2O}$$

Various organic acids can be obtained by the oxidation of sugars, all of which are aldehydes or ketones, and acids thus formed in plants lead on to the synthesis of fats. The way in which the glycerine arises is not yet known, but it is in all probability derived from the sugars also. Fat is formed in ripening seeds from the sugars which are translocated into them,

$$\begin{tikzcd} & \text{Fatty acids} \arrow[dr] & \\ \text{Sugars} \arrow[ur] \arrow[dr] & & \text{Fats} \\ & \text{Glycerine} \arrow[ur] & \end{tikzcd}$$

When the seed germinates the breakdown of the fats to sugars occurs in the reverse manner, and is so rapid that some of the sugar passes over into starch.

$$\begin{tikzcd}
 & \text{Fatty acids} \arrow[dr] & & \\
\text{Fats} \arrow[ur] \arrow[dr] & & \text{Sugars} \arrow[r] & \text{Starch} \\
 & \text{Glycerine} \arrow[ur] & &
\end{tikzcd}$$

Fats have a higher heat of combustion than the polysaccharides and consequently when they are oxidized they yield an even greater amount of energy per unit weight of material consumed. The change from sugar to fat must thus involve the uptake of further energy, but this is derived from respiratory energy not from light, since fats may be formed in parts of the plant which do not photosynthesize. The conversion of sugars to fats does not, therefore, increase the total amount of energy within the plant, but merely redistributes it.

EXPERIMENTAL WORK

Exp. 15. DISAPPEARANCE OF STARCH FROM DARKENED LEAVES

a. Take a pot plant of *Pelargonium* or *Sparmannia* which has been exposed to bright light for several hours. Detach one leaf and test for starch by the iodine method (**exp. 4**); detach a second leaf and place with the petiole dipping in water. Leave in a dark room or cupboard, with the whole plant by its side. At the end of two days make a starch test upon the other detached leaf and upon a leaf which has been left on the plant. The detached leaves will both contain abundant starch, the leaf attached to the darkened plant will have none.

b. In another leaf of the above plant sever a large vein without otherwise damaging the tissues. When the plant has been in the dark for two days, detach the whole leaf and test for starch. The part beyond the cut in the vein will still contain starch, but the rest of the leaf will not.

Exp. 16. STARCH IN TUBERS, &c.

a. Cut a potato tuber in half, and smear a few drops of iodine on the cut surface. A blue-black colour is formed.

A similar result may be obtained with crocus corms, iris rhizomes, and many bulbs.

b. Cut a section of a piece of potato tuber, mount in dilute glycerine, and examine under the microscope. The small cells are full of large shiny starch grains, with their characteristic concentric layers. Place a drop of iodine at one side of the cover-slip, and draw through by touching the far side with blotting-paper. Watch the starch grains become bright blue and finally almost black.

Exp. 17. FORMATION OF GLUCOSIDES; PRULAURASIN IN LEAVES

Chop up a leaf of cherry laurel (*Prunus laurocerasus*) into small pieces and put into a flask or test-tube. Hang a moist strip of sodium picrate paper into the vessel, and fix by corking. Into a second flask or tube put an undamaged leaf and a similar strip of picrate paper.

After a short time the paper in the first tube will have changed through orange to red, but the second will show no change. The reddening is caused by prussic acid, which is liberated from the glucoside prulaurasin in the damaged leaves. In the undamaged leaves the glucoside is not split. Owing to the prussic acid formed crushed cherry laurel leaves will kill moths and other insects.

Exp. 18. AESCULIN IN TWIGS

Strip the bark from some young horse-chestnut twigs and boil with a little water in an evaporating dish. Filter off the liquid and then pour it into a relatively large amount of water in a large beaker. A blue fluorescent solution is formed which is characteristic of the glucoside aesculin. The fluorescence is increased by making the solution slightly alkaline.

Exp. 19. THE REACTIONS OF CELLULOSE

a. Pour some aqueous iodine solution (iodine in potassium iodide) into an evaporating basin (or clean white saucer). Immerse in the liquid a small piece of cotton-wool or a strip of good writing paper. Pour off the iodine and add a few drops of strong sulphuric acid. On adding the acid a blue colour will be given.

b. Put a piece of cotton-wool or good writing paper into a basin and add a drop or two of chlor-zinc-iodine. A blue colour will be produced as before.

Exp. 20. CELLULOSE IN THE PLANT

a. Split a number of lupin seeds in half, and put in a basin. Add a drop of iodine in potassium iodide, and a few drops of strong sulphuric acid. The cell walls in this seed are particularly thick, and so the blue coloration of their cellulose is easily seen.

b. Cut a section of the seed and mount in chlor-zinc-iodine. Examine under the microscope and notice that the cell walls have become blue and much swollen.

Exp. 21. REACTIONS OF LIGNO-CELLULOSE

a. Dip the end of a match-stick into a solution of aniline sulphate or aniline chloride. It turns a bright yellow.

b. Dip the end of a match-stick into an alcoholic solution of phloroglucin. When well soaked dip into strong hydrochloric acid. A bright red colour is produced.

It is interesting to perform these tests upon a strip of common newspaper; this is made from wood pulp not from cotton rags like paper of better quality.

Exp. 22. LIGNO-CELLULOSE IN PLANTS

a. Strip the bark from any woody twig, and treat the wood as in experiment 21 above. The characteristic colour of ligno-cellulose will be formed.

b. Cut sections of a young woody twig, and mount in the reagents. Notice the coloration of the woody tissues; the cellulose walls of other tissues do not react.

Exp. 23. REACTIONS OF FATS

Pour a little olive-oil (a liquid fat) into a test-tube and add a few drops of the red dye Sudan III. Shake well and notice that the oil takes up the red colour. Pour half the coloured oil into a second test-tube. To one tube add water and to the other alcohol. Shake, and when the liquids have separated again notice that the dye has remained with the oil. Compare the density of the oil with that of water and alcohol, by the relative positions assumed in the two cases.

Exp. 24. FATS IN SEEDS

Crush some linseed (seeds of flax) in a mortar, and transfer to a test-tube. One-third fill the test-tube with water and boil. Drops of oil (melted fat) will escape from the crushed seeds and rise to the surface of the water. When a layer of oil has been collected add Sudan III and shake. When the two layers separate again, notice that the oil is stained light red.

III

NITROGENOUS COMPOUNDS

THE CHEMISTRY OF THE PROTEINS

THE substances dealt with in the previous chapter possess only carbon, hydrogen, and oxygen in their molecules, but many important compounds are found which contain nitrogen in addition. The most important of these are the *proteins* and *amino-acids*, which are related to one another in somewhat the same way as polysaccharides and sugars, though there are several important differences. There are numerous amino-acids, but they resemble one another in possessing both acidic and basic groups in their molecules, so that they behave as weak acids towards strong bases, and as weak bases towards strong acids. Substances of this kind are called *amphoteric*, and amino-acids and proteins are the most important examples of the class. The simplest amino-acid is called *glycine*, or *amino-acetic acid*, and has the formula

$$NH_2CH_2COOH$$

The hydrogen of the carboxyl, $-COOH$, group is easily replaceable, or in other words is acidic, and reacts with an alkali as follows:

$$NH_2CH_2COOH+NaOH \rightarrow NH_2CH_2COONa+H_2O$$

The product of the reaction in this case is sodium amino-acetate. With hydrochloric acid the basic amino, NH_2, group forms a salt called glycine hydrochloride:

$$HCl+NH_2CH_2COOH \rightarrow NH_3Cl.CH_2COOH$$

The basic group of one amino-acid molecule can react with the acidic group of a second molecule to form a *dipeptide*, thus

$$NH_2CH_2COOH+NH_2CH_2COOH \rightarrow \underset{\text{diglycine}}{NH_2CH_2CO{:}NH.CH_2COOH}+H_2O$$

This reaction is a condensation, since water is removed from the amino-acid molecules, but it differs from the condensation which occurs in carbohydrates and is distinguished by the name of *peptide linkage.* The dipeptide thus formed is still amphoteric, possessing an amino and a carboxyl grouping, and is able to condense again in the same manner as before. Such linkages have been brought about experimentally, but the complex polypeptide molecule thus formed was then too inert to condense further.

Proteins are formed by the condensation of large numbers of amino-acids, including glycine and many others, such as tryptophane, tyrosine, and cystine, the last having sulphur in its molecule. The proteins are extremely complex substances and have not been synthesized artificially up to the present, but by means of appropriate enzymes they can be hydrolysed into their constituent amino-acids, and this occurs in the following stages:

PROTEINS.	Insoluble	in strong	salt	solutions.	Indiffusible.
METAPROTEINS.	Insoluble	,,	,,	,,	Indiffusible.
PROTEOSES.	Insoluble	,,	,,	,,	Indiffusible.
PEPTONES.	Soluble	,,	,,	,,	Diffusible.
POLYPEPTIDES.	Soluble	,,	,,	,,	Diffusible.
AMINO-ACIDS.	Soluble	,,	,,	,,	Diffusible.

Each substance, or rather group of substances, in this descending series is simpler than the one above, the amino-acids being the simplest and hence in all probability the starting-point of protein synthesis. The union of amino-acids in the peptide linkage is of great importance in the construction of protein molecules, but since artificial polypeptides are so inert, it is clearly not the only factor involved. Every protein contains a variety of amino-acids which possess between them many complex side-chains, and the part these play is not yet understood.

Classification of the Proteins. The structures of the individual protein molecules are still unascertained, owing to their peculiar properties and their immense complexity. The latter may be judged from the approximate empirical formula for edestin, which is probably one of the simplest of the proteins. It is

$$C_{622}H_{1020}N_{193}O_{201}S_4$$

which corresponds to a molecular weight of 14,530.

Owing to the lack of certain chemical knowledge the proteins are classified according to their solubilities, and four classes are commonly recognized as occurring in plants.

ALBUMINS.	Soluble in water and neutral salt solutions.
GLOBULINS.	Insoluble in water, but soluble in neutral salt solutions.
PROLAMINS.	Insoluble in water and salt solutions but soluble in dilute alcohol.
GLUTELINS.	Insoluble in water and salts, but soluble in dilute acids and alkalis.

The only proteins which have been at all thoroughly examined up to the present are those in seeds, where they are particularly abundant. It is not certain that the proteins which are known to exist in other plant organs belong to any of these classes, and those extracted from leaves certainly did not seem to do so. As far as present knowledge goes, however, the first two classes are by far the most abundant in plants. In the majority of seeds globulins are plentiful, and as they are probably only slightly soluble under protoplasmic conditions, they accumulate by much the same mechanism as starch. In the seeds of the cereals the globulins are scarce, and the insoluble prolamins form the protein reserves; they have, in fact, only been obtained from this source and are unknown in other plants or in animals.

Conjugated Proteins. The proteins are amphoteric substances like the amino-acids from which they are formed, and are able to unite with various other molecules in consequence. They may also form 'adsorption compounds' in virtue of their special colloidal properties which are explained later (p. 133). The substances with which they unite may be simple things, like potassium or hydrochloric acid, or they may themselves be complex organic groupings. Any compound formed by the union of a protein with another complex substance is called a conjugated protein, and in animals a considerable variety of such substances are known. In plants only a single class has been recognized up to the present, and its members are given the name of *nucleo-proteins*, since they are formed by conjugation with a complex organic acid called nucleic acid. Nucleo-proteins differ from one another according to the protein, or proteins, contained in them, nucleic acid being the same in all cases.

THE FORMATION OF PROTEINS AND AMINO-ACIDS IN PLANTS

There can be little doubt that proteins are formed in plants by the condensation of amino-acids, under the influence of suitable enzymes. This appears very probable from the fact that the hydrolysis of proteins by the same enzymes outside the plant leads to the production of amino-acids and nothing else. The position is much strengthened, moreover, by the observation that as the reserve proteins are formed during the ripening of seeds amino-acids are among the simpler substances which disappear. Other simple nitrogenous compounds are also removed, however, and they too may take a part directly or indirectly.

The Synthesis of Amino-acids. The origin of the amino-acids is somewhat more difficult to explain, and the problem is not made easier by the fact that they are never present in very large quantities. There seem to be at least two distinct methods of formation, one of which requires light, and therefore involves a photochemical reaction, and one for which light is not necessary. The light method goes on in leaves simultaneously with the assimilation of carbon, and is comparatively rapid. It has been shown, however, that if a supply of sugar is provided leaves continue to form proteins even in the dark, when no photochemical reaction is possible. In two sets of leaves, which were examined after a six days' experiment, those which had been kept in light had formed 152 gm. of new protein, while those which had been floated on a sugar solution in the dark had formed 49 gm. or about one-third as much. It is possible, therefore, that the photochemical reaction is concerned only with sugar formation, or in other words, is the photochemical reaction of carbon assimilation. This is by no means certain, however. Nitrogen first enters the plant in the form of inorganic nitrates taken up in solution from the soil, and it has been suggested that light is necessary to reduce the nitrate to an amino form. The reaction between potassium hyponitrite and freshly formed formaldehyde to form potassium formhydroxamate is photochemical, and can be represented as follows:

$$\mathrm{KNO + HCHO \rightarrow HCOH.NOK}$$

It can be brought about by the influence of ultra-violet light, and the same result can also be obtained starting with carbon dioxide and potassium nitrate. Potassium formhydroxamate hydrolyses in solution to the acid, which by loss of oxygen forms an amino compound.

$$\mathrm{HCOH.NOH - O \rightarrow HCOH.NH}$$

Newly formed or 'active' formaldehyde is able to react

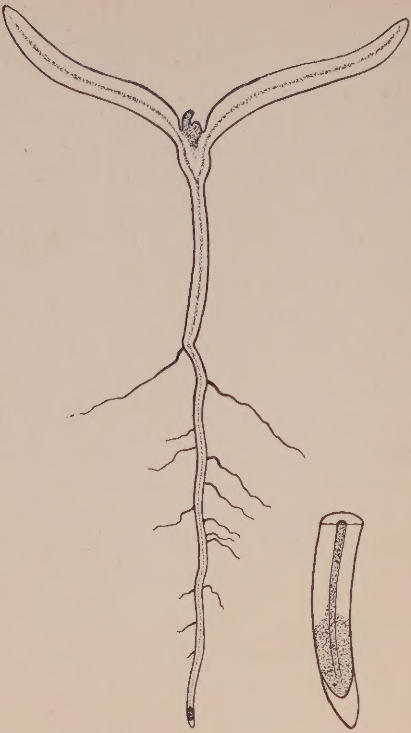


Fig. 17. THE DISTRIBUTION OF PROTEINS IN A SUGAR BEET SEEDLING

Regions rich in protein are shaded; the root tip is shown enlarged. (After de Vries, modified) with such a substance to give ring compounds, which rearrange to form simple amino-acids, such as glycine, the total change in this case being represented by the equation

$$\mathrm{HCOH.NH + HCHO \rightarrow CH_2NH_2COOH}$$

It is possible that amino-acids originate in plants in some such way as this, but it must be remembered that the expectation is based only on chemical reactions outside the plant. It was pointed out in speaking of carbon assimilation that formaldehyde has not been certainly identified in plants, and nobody has yet isolated formhydroxamic acid or its derivatives from a plant either. Although some people feel convinced that this must be the way in which vegetable proteins are synthesized, it is clear that the problem can only be settled finally by direct observations on plants themselves.

It seems highly probable that amino-acids and hence proteins are formed by a photochemical synthesis of some kind in leaves, but this is not likely to be the only method of formation, since proteins, as has been shown, also appear in leaves kept in the dark. The chemical mechanism of this 'dark synthesis' is even more obscure than the other, and we cannot say for certain in what parts of the plant it can go on, though perhaps the most probable guess is that it occurs in all living cells, which receive supplies of nitrates and sugars. Sugars can be oxidized to simple organic acids, and some of these are converted into amino-acids in the presence of ammonia, and it is possible that something of this kind is what happens in protein-forming cells. Nitrates are very scarce in leaves, but are usually comparatively plentiful in roots, and it has been said that the amount diminishes in a regular order from the fine rootlets upwards. Amino-acids are not plentiful anywhere in plants, but are usually present in the greatest concentration where nitrate is also most abundant, that is to say in the roots. The reducing power of the root cells due to the enzymes which they possess is also greater than that in the higher parts of plants, so it would appear that much of the nitrate is reduced to amino-acids very soon after it enters the plant and in subterranean organs as well as in those that receive sunlight. In this dark synthesis sunlight cannot be the source of the energy necessary to bring about the reaction, and we are obliged to presume that it is supplied by energy-liberating reactions going on inside the cells, such as occur in the complex sequences of respiration.

Protein Formation in Meristems. The distribution of proteins in a young plant is shown in fig. 17, and it will be seen that they are most abundant in the various meristems, or actively dividing tissues, where the cells have no vacuoles, and all their volume is occupied by protoplasm, containing a large proportion of protein material. These cells are continually increasing their number, and at the same time new material, including proteins, is formed in them. Conditions in meristematic cells seem to be particularly favourable to condensations in general, since many complex substances are built up in them in this way. Their walls are said to be very impermeable to water while they are young, and there are no large central vacuoles, such as occur in fully developed cells. It is probable that the proteins found so plentifully in meristems are synthesized in the usual way by the condensation of amino-acids, but the acids themselves are most probably formed in other parts of the plant, such as the leaves and roots, and being soluble substances a proportion of them at least is translocated to the meristems, where conditions are most favourable for the further changes to occur. Some of the proteins thus formed react with nucleic acid and form nucleo-proteins, which are abundant in the relatively large nuclei of meristematic cells.

NITROGEN ASSIMILATION

The formation of proteins involves a supply of carbohydrate and nitrogenous materials, and the origin of the former in plants has already been explained in speaking of carbon assimilation. Something may now be said of the assimilation of nitrogen, which differs in many respects from that of carbon, and so presents its own series of problems. In one respect, however, the two processes show a striking similarity. It has been mentioned that carbon dioxide is probably the only carbon compound taken up by autotrophic plants, and while the restriction is not quite so narrow in the case of nitrogen compounds, there are large quantities of such materials in the neighbourhood of plants which are not affected by them at all.

Soil Nitrogen. In all ordinary cases nitrates, nitrites, and ammonium salts seem to be the only forms which are directly available, and solutions of these are taken in by the roots, ramifying through the soil. As far as is known at present the penetration of these ions into the roots does not differ from the action of other inorganic ions, and it will be more convenient to deal with this stage of the process in a general way, taking all these substances into consideration. This is done in Chapter VI.

When the nitrates have entered the root cells chemical reactions begin to take place, with the result that they are reduced to nitrite and eventually to amino compounds. It is often stated that nitrates are more readily assimilated than the other forms, though it might seem curious since more energy is required for their reduction. Ammonium salts are used for fertilizing soils with very satisfactory results, but there are always bacteria present in the soil which slowly convert the ammonia into nitrate form, so that the nitrogen may enter the plant as nitrate even in this case. Experiments have been done with sterile cultures, and in these instances nitrates were usually, though not always, found to give the best results, but even here a difficulty arises. Ammonia is basic, and when it is taken up rapidly from a culture the solution becomes acid, whereas if a nitrate is used the solution becomes alkaline, and it is possible that the unequal growth produced in the plants is due to secondary causes of this kind, rather than to direct differences between the NH_4 and NO_3 groups. It is probable, therefore, that any of these simple inorganic compounds of nitrogen can enter the plant and eventually give rise to amino-acids, and since nitrates are more abundant than nitrites and ammonium compounds in most soils, they are probably the most important under natural conditions.

In addition to these simple substances there is a great deal of organic nitrogen in fertile soils, very much more, in fact, than there is of the inorganic. This is contained in the dark-coloured *humus*, which is formed by the decay of plant remains, and contains nitrogenous and other organic compounds of all stages of complexity. The more elaborate of these substances are usually indiffusible, and consequently do not penetrate into the roots of plants. It is easy, in fact, to grow normal green plants in culture solutions containing inorganic salts and no organic material whatever, so that humus and its constituents are not a necessity. On the other hand, this does not prove that the simpler soluble humus compounds are not taken into plants growing in rich soils, where they are abundant, and this question has not yet received an answer.

Mycorrhiza. A great number of plants, particularly the heaths (*Ericaceae*), orchids (*Orchidaceae*), and many forest trees, grow in intimate association with certain fungi, which ramify within their roots and over the root surfaces. The fungi are unable to synthesize carbon and nitrogen compounds in the manner of a green plant, but absorb soluble organic substances which contain these elements, from humus, from the host plant, or from other available sources. The mycorrhizal fungi thus receive carbon and nitrogen in organic forms from the soil, and part of this material finds its way eventually into the roots of the aerial plants. Some plants, indeed, only flourish when mycorrhiza is present at their roots and are unable to grow if planted in sterilized soil in which there is no suitable fungus to infect them.

The case of forest trees illustrates the relationship between the host and fungus very well. The soils in which they are growing commonly holds very little inorganic nitrogen, owing to the immense drain upon it and the comparatively slow rate of restitution. Organic nitrogen, derived from the decay of fallen leaves, is on the other hand very plentiful, and under these conditions the fungus thrives, absorbing a great deal of material, some of which finds its way finally into the host. If for any reason the soil relationships are reversed and inorganic nitrogen is the more abundant, the trees synthesize their proteins from this source in the usual way, and the fungus becomes a direct drain upon the process. It has been observed in many cases of this kind that the fungal threads disappear, being dissolved by the enzymes of the root cells, though in the absence of inorganic nitrogen they may persist more or less indefinitely.

Atmospheric Nitrogen. About four-fifths of the atmosphere which surrounds the aerial parts of plants consists of free nitrogen, a large amount of which is also dissolved in natural waters. The question at once arises, therefore, whether the uncombined element is taken up and converted to proteins in plants, or whether it is inactive in plant processes. The problem is of considerable importance in agriculture and for this reason it was investigated in the early days of agricultural science. The method employed was to grow plants such as wheat seedlings in pots of soil deficient in nitrogen, or in sand which was moistened with solutions containing only mineral nutrients. Air was allowed free access, and in the majority of cases it was found that seedlings thus grown contained no more nitrogen than a sample of the seeds from which they were derived. The only accessible nitrogen was that present in the air, and the seedlings were not able to elaborate any of this into organic material. Leguminous seedlings such as peas and clover when grown in soil exhibited a marked difference from the usual rule, and appeared able to assimilate atmospheric nitrogen in considerable quantities. Experiments were also performed with crop plants growing in farm fields, parts of which had become depleted of their soil nitrogen, and from these similar results were obtained. As an outcome of these experiments most people were convinced that the majority of plants do not assimilate nitrogen from the air as a free element, though the contrary idea has been revived from time to time. More elaborate and critical experiments have been done of recent years and their result has been to confirm the early work.

The Root Nodules of Leguminosae. The peculiar behaviour of leguminous plants remained for some years a mystery, but was eventually discovered to depend upon the close association of these plants with bacteria living in their roots. If the roots of a young bean plant (fig. 18) are carefully washed and examined they will be found to be swollen in various places into small excrescences or nodules, which have been formed in the following manner. The bacterium, *Bacillus radicicola*, is generally found in fertile soils, and almost invariably where beans and other legumes have grown recently. It can exist in a number of different forms, but is usually to be found as small spheres, or *cocci*, which may develop cilia and move about to a limited extent. If a coccus comes into contact with a root-hair of one of the *Leguminosae*, it becomes attached and multiplies upon the surface of the hair, the wall of which is softened, so that the bacteria find their way into the cell. Inside the

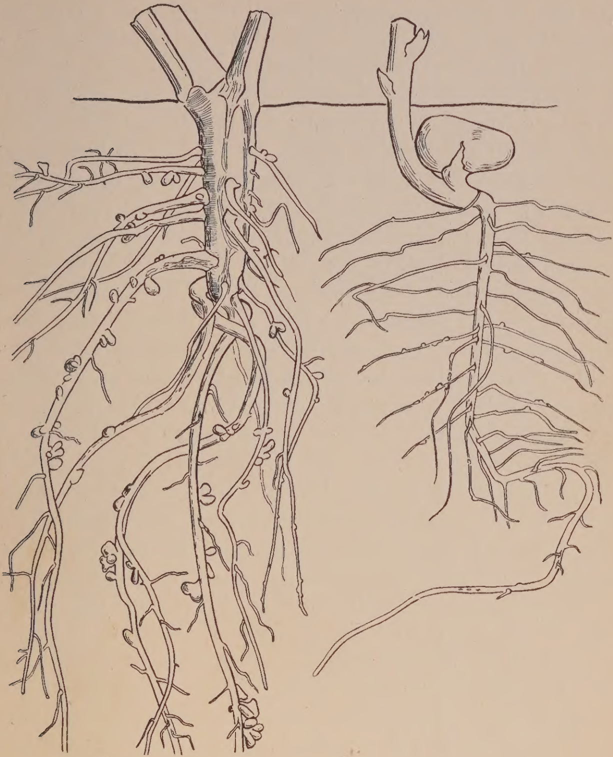


FIG. 18. ROOT SYSTEM OF *Vicia faba* SHOWING NODULES
Right, young plant with nodules just beginning to form; left, mature plant with nodules well developed. Reduced

plant the bacteria change their shape to a short rod and penetrate as far as the inner cortex of the root, but beyond this they never seem to go. Round the infected region the cortical cells divide repeatedly, while the cells which contain the bacteria considerably increase their size, so that a mass of new tissue is formed, which becomes visible externally as the nodule. In their final position the bacterial rods take on yet another shape, many of them branching into the form of a Y or a T, thus completing the cycle of changes shown in the accompanying diagram (fig. 19 A).

The bacteria which become immured in this way in the roots of suitable host plants convert uncombined nitrogen into simple nitrogenous compounds and finally into proteins. It is not certain that they do this while they exist independently in the soil, although it seems very probable, and there are bacteria which certainly do. When the root-nodule is being formed, the vascular tissues of the root form new branches, which envelop the infected region (fig. 19 C), and through this channel an exchange of soluble substances goes on between the host and the bacteria. Sugars, which the bacteria do not form themselves, pass from the autotrophic plant into the nodule, while soluble nitrogenous substances pass in the reverse direction.

The most notable result of this combination of organisms is the fixation of quite large amounts of atmospheric nitrogen, which reach the roots through the air spaces of the soil. The quantities involved are so great that if the plants rot upon the ground, or are ploughed into it, there is an appreciable increase in the combined nitrogen store of the soil, with a corresponding increase in fertility. The advantage of growing vetches or clover, which are both members of the *Leguminosae*, upon impoverished soil has been known to farmers of all ages, and was recorded by Theophrastus, the earliest botanical writer. The explanation of the gain is, on the other hand, not much older than the present century.

Nitrogen Fixation by other Organisms. The combination of nitrogen by the bacteria associated with the *Leguminosae* is the best known, but not the only, case of its kind. It has recently been shown that some at least of the mycorrhizal fungi take up nitrogen in this way as well as by absorbing its simpler organic compounds. More important than these, however, are certain bacteria living freely in soils,

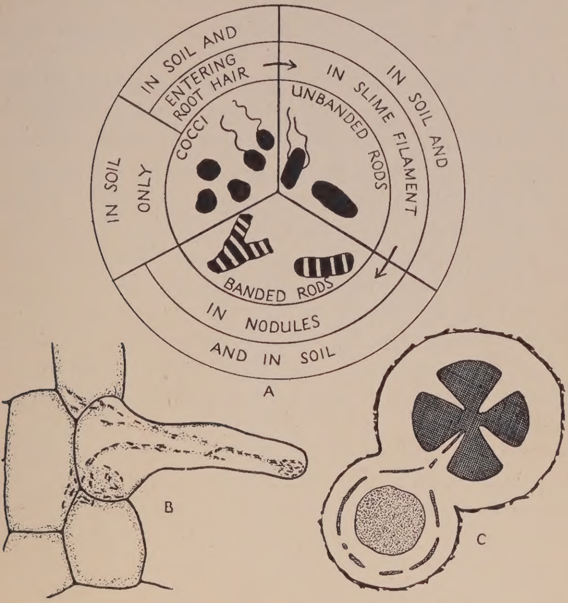

IN SOIL AND
ENTERING ROOT HAIR
COCCI
IN SLIME FILAMENT
UNBANDED RODS
IN SOIL AND
IN SOIL ONLY
BANDED RODS
IN NODULES
AND IN SOIL
A
B
C

FIG. 19

A. Diagram of the life cycle of *Bacillus radicicola*

B. Bacteria of the same species infecting a root hair of *Phyllocladus* ×500

C. Transverse section of a root of *Vicia faba* passing through a nodule
Bacterial region shaded with dots; vascular tissue cross hatched
(*B*. redrawn after Spratt. M.S.)

particularly *Azotobacter chroococcum* and *Clostridium pasteurianum*. These organisms are found in practically all soils, and are much more widely spread than *Bacillus radicicola*, which is only abundant in the neighbourhood of legumes.

Azotobacter may cause the fixation of considerable quantities of free nitrogen, and although it does not enter into organic association, or *symbiosis*, with plants, the amount of nitrogen it takes up is greatly increased by the presence of other unicellular organisms, which do not affect the nitrogen themselves. The reason for the increase is not at all clear, and probably differs according to the nature of the second organism which is introduced. The amount of nitrogen fixed is also dependent upon the conditions to which the bacterium is subjected; there must, for example, be a supply of sugars, but not a great deal of nitrate, or the latter is assimilated and nitrogen fixation comes to a standstill.

Clostridium is also affected by the presence of auxiliary bacteria, but in a different way from *Azotobacter*. When isolated and grown in pure cultures, that is to say, in the absence of all other organisms, it only assimilates free nitrogen, when oxygen is rigidly excluded. In soils, however, or in the presence of certain protecting bacteria, oxygen has no inhibiting effect.

The chemistry of nitrogen fixation by all these organisms is at present unknown, but in each case the building of the proteins requires energy in the same way as their synthesis from combined nitrogen. This is derived from the breakdown of sugar, by oxidation in the presence of oxygen, or by fermentation in its absence. The actual amount of sugar required differs among the various organisms, thus *Azotobacter* fixes more nitrogen than *Clostridium* for each gramme of sugar decomposed, even when the latter is supplied with oxygen.

THE NITROGEN CYCLE

It will be clear from the preceding pages that the higher plant is only one out of many nitrogen transformers which exist in nature. The cycle of changes through which the element is continually passing is indeed very complex, but its main outlines can be fairly easily stated, and will become clear from an inspection of fig. 20, p. 89.

Nitrogen Fixation in Air and Soil. Many of the changes represented have been described already, and others, such as the transformation of plant to animal proteins, need no further comment. During thunderstorms small quantities of nitrogen may combine with oxygen or with the traces of hydrogen present in the upper air, under the influence of the high-tension electric discharges. In this way traces of nitric oxide and ammonia are formed, which, being very soluble, are easily washed down by rain to form nitrates and ammonium compounds in the soil. The amount of nitrogen turnover due to this cause is comparatively small, however, and by far the most important 'fixations' are those going on in the soil, the air spaces of which contain free nitrogen, so that *Azotobacter* and *Clostridium*, as well as *Bacillus radicicola*, exist in an atmosphere containing a large proportion of nitrogen just as much as an aerial plant.

Nitrification. The action of the nitrifying bacteria, *Nitrosomonas* and *Nitrobacter*, which also exist in all common soils, has already been described to some extent (p. 34). The nitrogen transformations which they bring about make them independent of light, since the reaction in each case releases free energy. In this respect they differ very markedly from the nitrogen fixing bacteria, which depend upon preformed organic matter for their energy supplies, and which without such supplies would no longer assimilate nitrogen, or continue any other vital activity.

Nitrosomonas oxidizes ammonium carbonate in the soil, and its oxidizing action is entirely specific, that is to say it will not oxidize other nitrogen compounds such as urea or nitrites, or even other ammonium compounds. *Nitrobacter* is similar in this respect, its action being limited to the oxidation of nitrites alone. A curious property of these two organisms is that neither tolerates the presence of gelatine, and since this is generally used as a medium for the cultivation of pure strains of bacteria, it was a long time before they could be isolated and their mode of life studied. Finally cultures were successfully obtained with silica jellies free from organic matter, and their absorption of carbon dioxide to form carbohydrates with the energy derived from ammonia or nitrite oxidation was then made clear. Light was found to be not only unnecessary but actually fatal to these organisms.

The Formation of Ammonia. The proteins of plant and animal remains which become added to the soil after the death of the organism pass through the processes of decay. These are brought about in the main by a group of bacteria, though other members of the soil population also play a part, and some of the later stages go on even under sterile conditions. The principal agents are those known as *Bacterium mycoides*, though this name includes not a single species, but rather a group of species, all rather similar.

In their energy relationships these bacteria differ from the nitrogen-fixing bacteria in an interesting way. If proteins are scarce and sugars abundant, it has been noticed that they cease to produce ammonia, and absorb nitrogen compounds like the others, energy being derived from the oxidation of the sugars. If proteins are present, on the other hand, they are decomposed by the bacteria, which become independent of sugars and simpler nitrogen compounds, deriving both their energy and materials from the single source. During this process more ammonia is formed than is absorbed by the bacteria, and the surplus is therefore released into the soil, where it forms compounds, principally the carbonate. Unlike most organisms, these bacteria are able to use combined oxygen, and will bring about oxidation of proteins or sugars in the absence of the free element, if compounds such as nitrates are present.

The three processes, leading to the formation of ammonium carbonate, nitrites, and nitrates in soils are closely connected, since the final product of each is the starting-point for a further reaction. They form, in fact, a linked series of reactions, so that the amount of each of these substances to be found in the soil depends upon the relative rates of the three reactions in the chain. Direct analysis shows that even when proteins are abundant ammonium carbonate and nitrites are only to be found in traces, though nitrates are somewhat more plentiful. From this it can be deduced that the formation of nitrates from nitrites is a comparatively rapid process, nitrite formation rather less rapid, and the formation of ammonium carbonate slower than either of the other reactions.

Denitrifying Bacteria. The principal living agent in the removal of nitrates from soils is the green plant, but there are also numerous bacteria which diminish their quantity. As already explained *Bacterium mycoides* behaves in this way when free oxygen is scarce and there is a good supply of easily oxidized organic material. These conditions also favour similar reactions on the part of numerous other bacteria found in soils, the nitrates being reduced to nitrites and eventually to ammonia. It is very rarely that such a state is found in ordinary soils, however, and one of the objects of cultivation is to keep the soil crumbly with plenty of air spaces and hence well aerated. In agricultural land, therefore, and even in the majority of natural soils denitrification by bacteria is not at all rapid, although it may become so in very rich or waterlogged land.

Oxidizing Bacteria. The same conditions favour the action of bacteria which oxidize decaying organic matter, with the liberation of a certain amount of free nitrogen. Their activity in ordinary soils is very slight, but in sewage or manure heaps they may cause the loss of appreciable quantities of the element, by liberating it into the air. Since losses of this kind are a drawback in agriculture, the process has been extensively examined, but up to the present the nature of the chemical reactions has not been explained.

The Nitrogen Balance of the Soil. Since the amount of nitrogen in simple inorganic combination to be found in the soil is one of the most important factors in plant growth, it will be well briefly to summarize the processes which control it. The most important of those tending to increase the amount are the putrefactions of plant and animal remains, so it might easily be supposed that the addition of such substances to soils would lead to the formation of nitrites and nitrates in due course. This depends very largely, however, upon the relative amount of carbonaceous and nitrogenous substances present in the residues. If the material added has much nitrogen in protein and other complex forms, the putrefying bacteria will produce large quantities of ammonia, which is afterwards converted to nitrate. If, on the other hand, the material is poor in nitrogen, but supplies a good deal of carbohydrate, many soil bacteria increase their numbers owing to the energy they derive from the latter, and consequently draw upon nitrates already in the soil, and so impoverish it. Against this the nitrogen-fixing bacteria are also favoured by such conditions and slowly increase the nitrates at the expense of the carbohydrates. As a consequence of these and many other reactions going on at the same time, the ratio between

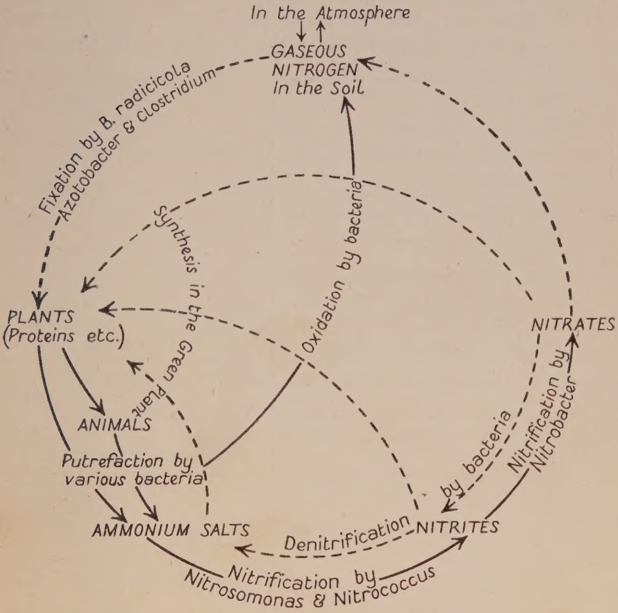

In the Atmosphere
GASEOUS NITROGEN In the Soil
Fixation by B. radicicola
Azotobacter & Clostridium
Oxidation by bacteria
Synthesis in the Green Plant
PLANTS (Proteins etc.)
ANIMALS
Putrefaction by various bacteria
AMMONIUM SALTS
Denitrification
NITRITES
NITRATES
by bacteria
Nitrification by Nitrobacter
Nitrification by Nitrosomonas & Nitrococcus

FIG. 20. THE NITROGEN CYCLE

Diagram to show the principal natural transformations of the element. The chemical compounds, etc., are shown in capitals; the names of processes and their agents in smaller type. The broken lines indicate reactions in which energy is used up (endothermic), and the continuous lines reactions in which energy is released (exothermic)

carbon and nitrogen is not easily altered, and usually has a numerical value of about 10 in damp soils such as are found in the temperate zone. The stability of this ratio is illustrated by the observation that addition of inorganic nitrates does not lead to a permanent enrichment of the soil unless carbonaceous material, such as wheat stubble, is added also.

The following list is a summary of the principal nitrogen transformations, which go on in fertile soils, together with the members of the soil population which cause them.

Name of Process	*Agents*	*Chemical Changes Involved*
Nitrogen Fixation	Bacillus radicicola Azotobacter chroococcum Clostridium pasteurianum Fungi of mycorrhiza	Free nitrogen→Proteins
Nitrification	Nitrosomonas Nitrobacter	$(NH_4)_2CO_3+3O_2 \rightarrow 2HNO_2+CO_2+3H_2O$ $Ca(NO_2)_2+O_2 \rightarrow Ca(NO_3)_2$
Putrefaction	'Bacterium mycoides', etc.	Proteins → Ammonia, &c.
Denitrification	'Bacterium mycoides', etc.	Nitrates → Nitrites → Ammonia
Oxidation	Various oxidizing bacteria.	Oxidation of organic compounds with liberation of free nitrogen

EXPERIMENTAL WORK

Exp. 25. Reactions of Proteins

Break an egg and collect the white in a cup. About half fill the cup with water, and stir briskly until an even solution of the egg albumin (a protein) is obtained.

a. Xanthoproteic test. One-third fill a test-tube with the protein solution and add about one-third as much strong nitric acid. A cloudy white precipitate will be formed. Boil; the precipitate will turn canary yellow; cool by holding the end of the test-tube under the tap, and then make alkaline by adding ammonia. The yellow colour will turn to orange.

b. Biuret reaction. Put some protein solution into a second test-tube; add about 2 c.c. of 40 per cent. sodium hydroxide, and one drop of 1 per cent. copper sulphate. A pale purple colour is produced.

c. Add a few drops of Millon's reagent to 2 or 3 c.c. of the solution of protein; on boiling, a brick-red precipitate or solution will be obtained.

Exp. 26. PRESENCE OF PROTEINS IN PLANTS

a. By means of the above tests examine the following plant materials for proteins: peas, potato peelings and centres, and flour.

b. Take a very young cress seedling and lay it upon a microscope slide. With a dropping rod cover the root and hypocotyl with Millon's reagent. Warm gently for several minutes over a flame or beaker of boiling water, adding water if necessary to prevent the reagent from drying up. Examine under a microscope. A reddish colour showing the presence of protein (and related substances) will be seen, especially at the root tip. If necessary the root may be crushed by pressing gently on the cover-slip with a pencil.

Exp. 27. ROOT NODULES

Sterilize enough good potting soil to fill a five-inch pot, by heating with an excess of water in a large metal dish or open saucepan. Keep at the boil for about ten minutes, and finally evaporate the water by more cautious heating. When dry enough cool and put into a well-cleaned pot. Prepare a second pot with untreated soil and sow several broad or runner beans in each. When the young plants are about ten inches high knock them out of the pots, and wash the soil away from the roots. Those grown in unsterilized soil will show numerous small nodules, whereas the others will have none.

IV

THE DECOMPOSITION OF SUGARS AND THE LIBERATION OF ENERGY

PLANT RESPIRATION

The Nature of Plant Respiration. In the preceding chapters it has been shown how sugars are built up into the complex substances of the plant body, but a certain proportion of them is always undergoing decomposition, and may never pass through the more complicated forms of material such as starch, cellulose, or protein. The breakdown is the process known as *glycolysis*, and is not restricted to the cells in which sugars are manufactured, because they make their way to all parts of the plant. The presence of varying amounts of sugars is, in fact, characteristic of all living cells, and there is every reason to suppose that glycolysis is always going on in them. At the same time carbon dioxide is given off and oxygen absorbed, often, though by no means invariably, in equal volumes. This exchange of gases, carried on by all actively living material, is easily observed and has been known for a long time under the name of *respiration*, and since the gaseous exchange is the same both in plants and animals, the same name has been given to the process in both kingdoms. It may be assumed in the case of plants that the carbon dioxide thus given off is derived from the sugars, which they manufacture so readily, and the usual way of summarizing these facts is by means of the following chemical equation:

$$C_6H_{12}O_6+6O_2 = 6CO_2+6H_2O$$

This method of statement has several shortcomings; for example a reaction which involves seven molecules is not likely to occur in a single step, and we have here no clue to the intermediate stages; moreover the amount of sugar which disappears for every molecule of carbon dioxide produced turns out to be much greater in some cases than the amount suggested by the equation. We know now that the chemical changes resulting from the breakdown of the sugars are extremely complicated, and that sugars themselves are not oxidized at all as appears from the equation, oxidation only occurring after the sugars have been transformed to other, and probably simpler, substances. This equation is not, therefore, a satisfactory summary of the chemical changes involved in plant respiration, and it is necessary to consider more fully what these changes are.

In the first place, there is probably a fairly large number of stages between the initial breakdown of sugar and the final emergence of carbon dioxide and the equivalent water. The substances at the two ends of this series of reactions are those which are also involved in photosynthesis, the final products of respiration being the initial reactants in assimilation, and vice versa. Nevertheless, the respiration sequence of reactions is not the same as the photosynthetic sequence in reverse order, the intermediate substances being entirely different in the two cases, and no photochemical reaction being involved in respiration.

It was believed at one time that the living protoplasm was constantly breaking down and that respiration consisted of an oxidation of this material. This is not the case, since protoplasm consists largely of nitrogenous substances and no corresponding nitrogenous waste products are formed by plants. The final external products of plant respiration are carbon dioxide and water alone, and they are produced from the oxidation of carbon and hydrogen, originally present as sugar molecules in the respiring material. Although carbohydrates alone are used up during respiration, the process only goes on in the presence of living matter, and a solution of sugar in contact with the oxygen of the air is perfectly stable and will remain unaltered for years, if it is protected from the action of living organisms such as moulds and bacteria. It follows, therefore, that we are not dealing with a simple series of chemical reactions which can proceed easily of its own accord once the initial substances have been brought together; besides the principal reactants, there must also be catalysts present to facilitate the reactions. In living cells all the necessities are provided by the protoplasm, which regulates and controls the respiratory changes, and even the rate at which they go on.

The Rate of Respiration. By putting a plant in the dark, photosynthesis is brought to a standstill, but respiration continues. Under these conditions carbon dioxide is slowly emitted and its formation can be shown by shutting the plant in a vessel and afterwards testing the enclosed air with lime water. If suitable arrangements are made (*see* **exp. 32**, p. 118) the rate at which the carbon dioxide is being formed can be accurately measured, and this may be called the *rate of respiration*. The respiratory rate could also be measured by the speed at which oxygen is absorbed, and these two measurements are all that are usually attempted, so that most of our knowledge of respiration has been built up from observations of this kind. The measurement of sugar losses and other changes in respiring plants is much more difficult, and has not yet been achieved on any large scale.

The actual rate at which respiration goes on depends upon a large number of factors, the most obvious and probably also the most important being

1. The state of the respiring protoplasm.
2. The concentration of respirable sugar.
3. The concentration and activity of the necessary enzymes.

Temperature, acidity, the presence of certain salts, and the amount of moisture all affect the rate of respiration, but their action is probably through one or other of the three factors given above. The activity of enzymes, for example, depends upon temperature, acidity, and the presence of certain activating and depressing substances, often of a simple inorganic nature; the acceleration of zymase action brought about by alkaline phosphates is an example.

Respiration throughout the Life Cycle. The state of a living protoplast is subject to frequent changes, but during the life history of the plant to which it belongs a slow sequence of alterations goes on, and can easily be recognized. Each protoplast has its own development, so that different cells or groups of cells in different parts of a plant are not all at the same stage of their growth, and it is possible to isolate tissues from a single plant which are in different phases of protoplasmic development. Such tissues differ from one another in their rates of respiration, and four stages, depending upon the condition of the protoplasts, can be recognized:

1. Embryonic, e.g. meristems or germinating embryos.
2. Mature, e.g. functional leaves, etc.
3. Senescent, e.g. ripening fruits.
4. Dormant, e.g. dry seeds.

A single cell may pass through several of these phases, and its mechanism, depending upon the condition of its protoplast, changes as it does so. The passage from one stage to the next is not sudden, however, but occurs as a gradual drift which continues throughout the life cycle. This drift is reflected in the rate of carbon dioxide emission, or rate of respiration. If the plant as a whole is considered, the respiratory index, i.e. the number of milligrams of carbon dioxide produced per hour, per gramme of dry weight, varies in the manner shown in fig. 21. During germination and the early development of the young seedling, which comprises the first or embryonic stage, respiration mounts rapidly to a maximum from the slow rate characteristic of the resting seed (dormant stage). The highest respiratory index of the plant's life is reached at the end of the embryonic stage, and thereafter, during the period of maturity, the index gradually declines. A disadvantage of this method of measuring the rate of respiration is that the unit of dry weight includes greater and greater amounts of inert, i.e. non-respiring matter, as time goes on, and the plant comes to contain an increasing proportion of cell wall and other non-living material. If the respiratory index of the stem apex, which never contains a large proportion of inert substance, is examined instead, it is found that the

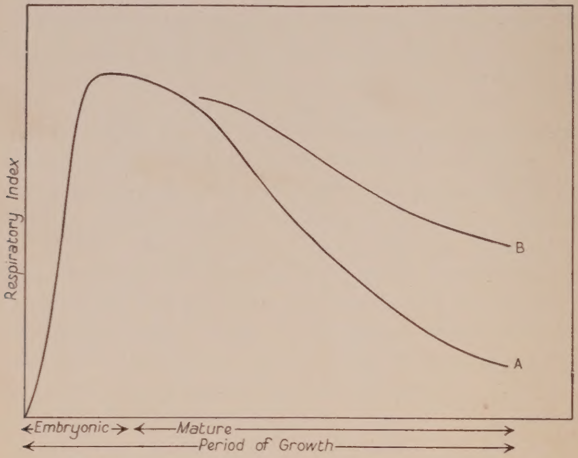


FIG. 21. Graph of the respiratory index (= milligrams of carbon dioxide emitted per hour per gm. of dry weight) throughout the life cycle of an annual plant. *A.* Respiratory index of whole plant, *B.* of stem apex. Diagrammatic

decline is less rapid, but does not disappear completely (*see* fig. 21 B), so that although the cells at the apex remain alive during the whole of the period represented, they pass gradually from their embryonic condition to one of maturity, in which they exhibit the slowly declining rate of respiration characteristic of this stage.

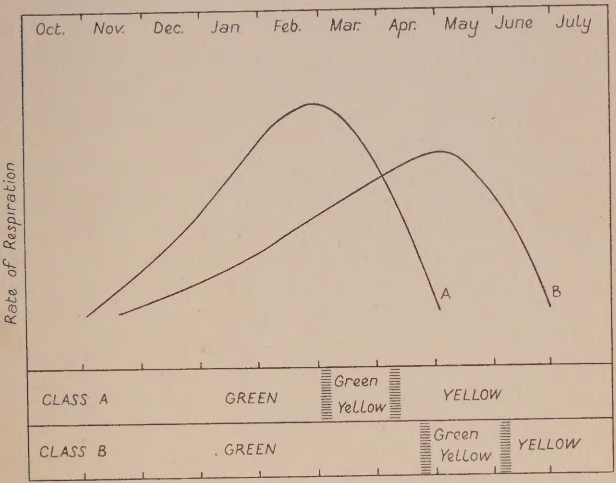


FIG. 22. Graph of the rate of respiration of two classes of apples stored at 2.5° C., with the colour of the apples indicated below. (After Blackman and Parija, modified)

The senescent state is best illustrated by tissues such as those present in ripening fruits, stored tubers, and yellowing leaves. If leaves are detached from the parent plant and prevented from forming further carbohydrates by being kept in the dark, they also pass into this condition, but the length of time taken to go through the senescent stage is much shorter in such cases; it is longest in tissues such as potato tubers, while fruits kept in cold storage occupy an intermediate position. In all these cases, however, respiration follows a characteristic course, the rate first rising from the comparatively low value reached in the mature stage, and after a longer or shorter period sinking to a low level again. The course of respiration of two different sorts of apples kept at a low temperature in the dark is shown in fig. 22. Class B, reaching its maximum respiration about two months later than class A, passes through its senescence more slowly, or in other words is a better keeping apple.

The course of respiration during the starvation of mature detached leaves can be represented diagrammatically as in fig. 23. When first placed in the dark, their respiration is comparatively rapid and falls gradually to a low value. After an interval, varying in length according to species, a marked acceleration sets in, lasting for a period roughly equal in duration. By the time this has happened the protoplasts of the leaf cells have passed, much more rapidly than in normal circumstances, from the mature, into and through the senescent stage. The nature of the protoplasmic changes is discussed more fully in the next chapter.

The state of dormancy is characterized by the extreme inertness of the protoplasm, and, correlated with this, the rate of respiration is so extremely slow that it may become impossible to measure the carbon dioxide emitted. This is the converse case of the very rapid respiration associated with the great protoplasmic activity during germination, and it may be said in general, that the more active the protoplasm, the higher the rate of respiration.

Respiration and Glucose. Several sugars are present in respiring cells, and the rate of respiration depends upon the concentration of the *respirable* sugar, which, there is now good reason to believe, is a form of glucose. The early part of the starvation curve given in fig. 23 shows a decline in respiration rate, and simultaneously with this fall there occurs a loss of sugar of all kinds. This does not mean that all sugars are directly respirable, since they are readily converted into one another in the plant, and it has in fact been shown that during periods of senescence there is a close connexion between the concentration of *hexose* sugar and the respiration rate. Similarly, in those cases which have been examined, hexose concentration varies during the successive stages of the plant's life history in the same manner as the rate of respiration, but there is no connexion of this kind between respiration rate and sucrose concentration.

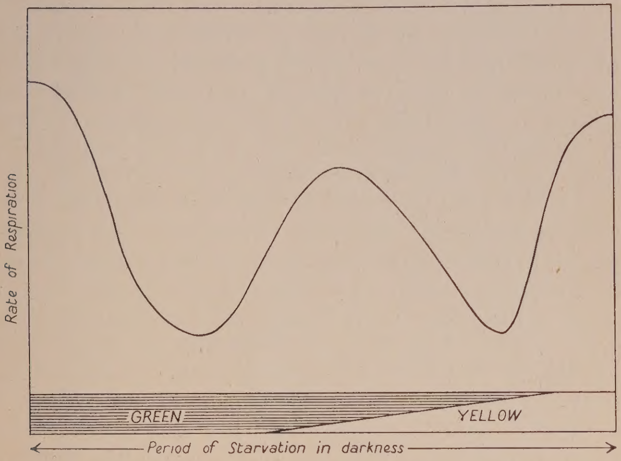


FIG. 23. Rate of respiration of mature detached leaves during starvation at about 20° C. The final rise in the rate of carbon dioxide emission is due to the multiplication of bacteria upon the disintegrated tissues. The proportions of the leaves appearing green and yellow are indicated in the lower strip. Diagrammatic

These experiments do not distinguish between the two hexose sugars common in the plant, namely glucose and fructose. That glucose and not fructose is the respirable sugar, is suggested by the fact that rapidly respiring fungi were found to use up all the glucose in a mixture of the two sugars, whereas fructose disappeared more gradually, being first converted into glucose.[1]

Any substance which may give rise to glucose in the plant cells may be regarded as a potential source of respirable material; such conversions are naturally most prominent during periods of starvation and senescence, when synthesizing tendencies are reduced to a minimum. In the earlier stages of growth condensation processes are active, and lead to the formation of polysaccharides and other complex bodies. In starvation or senescence the opposed process of hydrolysis becomes predominant, with the result that substances such as starch and sucrose are broken down to their constituent hexoses, chief amongst which is glucose. The hydrolyses of these two substances are usually the first to occur; at a later stage, indicated by the rising respiration rate, other glucose compounds, such as the cyanogenetic glucosides, are also broken down, and cause a temporary increase in the available glucose, leading on to the temporary increase of respiration.

The Action of Oxygen. One of the most important features of respiration is the fact that it includes a process of oxidation, so it is not surprising to find that the rate of respiration depends upon the amount of oxygen available. Respiration rate is fast in high concentrations, and drops gradually with diminishing partial pressure of the gas. In many cases the volume of oxygen absorbed in a given time is equal or approximately equal to the volume of carbon dioxide given out simultaneously, and this is sometimes expressed by saying that the respiration coefficient,

---

[1] Evidence has recently been brought forward which suggests that fructose is often respired even more readily than glucose.

CO_2/O_2, equals 1. This is only true, however, within certain limited conditions; the oxygen concentration, for example, must not be too high or too low. At low concentrations of oxygen, respiration rate, as measured by carbon dioxide emission, no longer falls with a further reduction of concentration; but below a certain value, varying with the plant and its condition at the time of measurement, the production of carbon dioxide actually increases again, as oxygen becomes scarcer. There is then an important difference from the state of affairs in higher oxygen concentrations, since the absorption of oxygen no longer keeps pace with the production of carbon dioxide, and at these low concentrations the highest rate of carbon dioxide production actually occurs in the complete absence of oxygen. It is clear that in this extreme case, respiration can no longer include the oxidation stage, which characterizes it in the presence of free oxygen, and the plant is then said to be respiring *anaerobically*, as distinct from its normal or *aerobic* respiration. Throughout the range of oxygen concentration in which carbon dioxide production is falling off (0 to about 5 per cent.), anaerobic respiration is steadily decreasing, while aerobic respiration only increases very gradually; finally anaerobic respiration ceases entirely, and with abundant oxygen respiration becomes entirely aerobic. The amount of oxygen absorbed is equal to the amount of carbon dioxide formed by aerobic respiration, and it will be seen that the value of the respiratory index must, therefore, differ at different oxygen concentrations, and will only be equal to unity when the concentration is high. It is also liable to changes depending upon the previous history of the respiring material.

Anaerobic Respiration. Anaerobic respiration has a number of features in common with alcoholic fermentation in yeast, and the resemblances between the two processes are in fact so striking that they have been considered more or less identical, but this turns out to be too simple a view of the case.

It has been shown that the net result of respiration in the absence of oxygen can be represented approximately by the equation

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

which is also the equation for alcoholic fermentation. When seeds were allowed to germinate in an enclosed atmosphere of hydrogen, carbon dioxide was given off, causing an increased pressure in the sealed tube. At the same time alcohol was found inside the seeds, corresponding roughly in amount to the quantity of carbon dioxide produced, and a similar formation of alcohol has also been ascertained in stored apples.

The equation shows that although no oxygen has been added from outside there has been a redistribution of the oxygen already present in the sugar among the carbon atoms in its molecule, with the result that the carbon finally found in the carbon dioxide has been oxidized at the expense of the carbon in the alcohol. These changes have not occurred in a single step, but only as a result of a long series of reactions, some of which can be described. Glucose exists in several forms, some of them more active than others, and the first step probably consists of the transformation of a less active into an 'activated' form. The activated glucose molecule, $C_6H_{12}O_6$, is broken down, probably into equal halves, and this is the process which has previously been mentioned as glycolysis.[1] The remaining stages leading on to alcohol and carbon dioxide may include the formation of pyruvic acid and acetaldehyde, as in alcoholic fermentation by yeast.

All plants are capable of carrying out anaerobic respiration, and most of them can be kept for many hours in the complete absence of oxygen without serious injury. Upon return to a normal atmosphere, aerobic respiration is resumed, though it often continues for some time at an increased rate, and only later returns to its original value. There is, however, no permanent effect upon the protoplasm, and once the initial respiration rate has been resumed there is nothing to indicate the unusual treatment of the plant. Nevertheless, the withholding of oxygen must not continue too long, since in anaerobic respiration the sugar supplies of the plant are used up very rapidly, so that starvation may result, in addition to which the internal accumulation of alcohol will eventually become poisonous. The majority of plants do, therefore, require oxygen for their normal method of respiration, but there are a few which can exist almost indefinitely in its absence, and some which are even poisoned by it. Examples of the former are some of the soil bacteria such as *Bacterium mycoides*, and these are sometimes distinguished by the name of *facultative anaerobes*, but this name may also be made to cover all ordinary plants, since they can endure anaerobic conditions for a time. Those which, like the tetanus bacillus, are killed by oxygen, are described as *obligate anaerobes*.

---

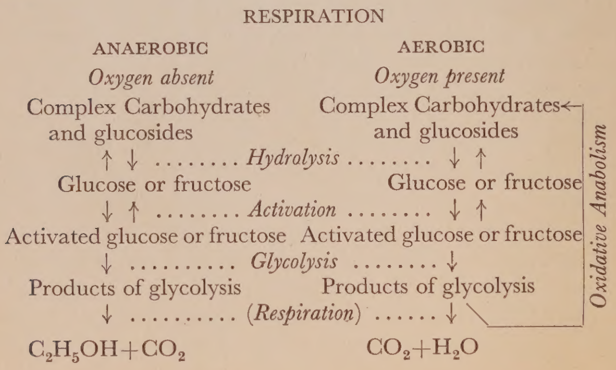
[1] The active sugar may be active fructose instead of an active glucose.

Aerobic Respiration. The two forms of respiration are not completely independent processes. In both of them a hexose is the substance used up, and the initial stages of its decomposition are probably the same whichever form of respiration is going on. It is thus possible to give the same name, *glycolysis*, to them in both cases, and it is not the hexose molecule which is oxidized, but the products formed from it by glycolysis. In the absence of free oxygen these are all converted into ethyl alcohol and carbon dioxide, but if oxygen is present oxidation of these products occurs, with the result that carbon dioxide and water are formed instead. If the disappearing hexose was quantitatively oxidized to these substances, the respiration equation

$$C_6H_{12}O_6+6O_2 \rightarrow 6CO_2+6H_2O$$

would be satisfied. This seems to occur in some cases, but in others the amount of sugar which is lost is much greater than is indicated, while still others are known in which it is less. Apples ripening in storage have been found to show an unexpectedly high rate of sugar loss, but no waste products have been identified to account for the missing carbon. It seems likely, therefore, that some of the products of glycolysis are built back into sugars or more complex carbohydrates, such as already exist in the plant. This building back only occurs in the presence of free oxygen, and since it is also an upgrade, or anabolic process, it has been called *oxidative anabolism.* It presents a marked contrast to other anabolic processes, such as photosynthesis, which involve the elimination of oxygen at one stage or another.

The downgrade, or *katabolic,* reactions going on in living cells and included under the title of respiration present a complex picture even when viewed only in outline, and it seems likely that the increase of knowledge will increase its complexity rather than diminish it in the near future. The salient features as at present known, may be summarized as follows:



RESPIRATION

ANAEROBIC		AEROBIC
Oxygen absent		*Oxygen present*
Complex Carbohydrates and glucosides		Complex Carbohydrates← and glucosides
↑ ↓	*Hydrolysis*	↓ ↑
Glucose or fructose		Glucose or fructose
↓ ↑	*Activation*	↓ ↑
Activated glucose or fructose		Activated glucose or fructose
↓	*Glycolysis*	↓
Products of glycolysis		Products of glycolysis
↓	*(Respiration)*	↓ ↘
$C_2H_5OH+CO_2$		CO_2+H_2O

Biological Oxidations. It is usual to think of oxygen as a very active element able to combine with a large number of other substances. Its activity differs enormously, however, according to its state, being much less in the molecular condition than in the atomic. In the atmosphere oxygen exists as molecules, described by the formula O_2, and these are quite unable to bring about many of the oxidations which occur in living cells. It has been found that molecular oxygen, which is entirely free from moisture, will not even oxidize carbon monoxide (CO) to carbon dioxide (CO_2). Molecular oxygen is in fact only effective in the presence of some very easily oxidized substance, such as hydrogen, and it is probable that water, which is present in all living cells, is at the basis of many biological oxidations. If some substance which absorbs or unites with hydrogen very readily is also present, hydrogen may be removed from water molecules, leaving free atomic oxygen, which is much more active than the molecular oxygen which enters from the air.

An inorganic model is afforded by palladium, which absorbs hydrogen very readily. It has been found that if this substance is brought into the presence of water and molecular oxygen, it is able to remove hydrogen from the water, forming the compound PdH_2, and liberating atomic oxygen.

$$Pd+H_2O \rightarrow PdH_2+O \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

In addition, the molecular oxygen reacts with the hydrogen in the PdH_2, forming water and liberating a further free atom of oxygen.

$$PdH_2+O_2 \rightarrow Pd+H_2O_2 \rightarrow Pd+H_2O+O \ldots\ldots\ldots\ldots\ldots (2)$$

Since the release of the free atomic oxygen involves the combination of hydrogen with some other substance, this process is called *oxido-reduction.*

There is good reason to believe that many oxidations taking place in animal cells occur in this manner, though the substance which replaces palladium has not yet been definitely ascertained.

If the dye methylene blue is added to tissues in which oxido-reduction is going on, it takes up hydrogen and becomes colourless, and the occurrence of the reaction can thus be easily detected. It has been found more difficult to discover whether oxido-reduction goes on in plant cells, because most of them do not happen to reduce methylene blue, or any similar hydrogen acceptors, which have yet been tried. In potato tubers, however, the reaction has been definitely discovered, and also in yeast.

The Respiration Enzymes. A large number of enzymes are involved in the various stages of respiration, and the activity of any one of them affects the rate at which respiration goes on. Even the hydrolysing enzymes, which break down the complex polysaccharides to sugars, affect the rate of respiration by determining the amount of hexose present to be respired. There is also a long sequence of cell catalysts promoting the subsequent stages of both aerobic and anaerobic respiration. The enzyme *zymase* was first discovered in yeast, where it was found to bring about the process of alcoholic fermentation, but it has since been discovered in all plants and their individual parts in which it has been sought. There seems no doubt that this enzyme enters into both anaerobic and aerobic respiration, since it is responsible for glycolysis.

A great variety of *oxidizing enzymes* have been found in plant cells, each of them able to bring about the oxidation of its own specific substrate. The enzymes grouped as *oxidases* add oxygen to a number of substances in the presence of the free element. It is probable that plant oxidases are not single substances, but are rather mixtures containing an organic peroxide, formed from *catechol* or one of its compounds, which are found widely distributed in plants, by the addition of atmospheric oxygen. Two enzymes are supposed to be concerned in the so-called oxidases, one an *oxygenase* which catalyses the formation of the organic peroxide from catechol, and another called *peroxidase* transferring the oxygen from the organic peroxide to the final substrate, which is usually a phenol, i.e. an alcohol related to benzene. The whole scheme may be represented as follows:

$$\begin{array}{c}\text{Catechol}\\\text{Compound}\end{array}+\begin{array}{c}\text{Atmospheric}\\\text{Oxygen}\end{array}\xrightarrow{\text{(OXYGENASE)}}\begin{array}{c}\text{Organic}\\\text{Peroxide}\end{array}$$

$$\begin{array}{c}\text{Organic}\\\text{Peroxide}\end{array}+\text{Phenol}\xrightarrow{\text{(PEROXIDASE)}}\begin{array}{c}\text{Catechol}\\\text{Compound}\end{array}+\begin{array}{c}\text{Oxidized}\\\text{Phenol}\end{array}$$

All plants possess peroxidases, but only some possess the oxygenase, and those without it can only bring about the oxidation of phenols, if some peroxide, such as hydrogen peroxide, is added to them. Hydrogen peroxide without the enzyme will not bring about this reaction.

The relation of these enzymes to aerobic respiration is still uncertain because we do not know what substances are formed during the oxidation stages of the process. The nature of the enzymes themselves and the ways in which they act are dealt with in the next chapter (p. 137).

The Energy Liberated during Respiration. By far the most important aspect of respiration is the release of energy which it involves. The greatest part of the total energy thus set free is liberated during the oxidation stages, but even in anaerobic respiration a small amount is released; this can be indicated by the equation

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH+2CO_2+9{,}400 \text{ cals.}$$

In aerobic respiration the energy derived from an equal amount of glucose is much greater and the corresponding equation is

$$C_6H_{12}O_6+6O_2 \rightarrow 6CO_2+6H_2O+677{,}000 \text{ cals.}$$

Protoplasm only remains in a living condition as long as there is a continuous energy release going on within it. In anaerobic respiration comparatively little energy is obtained, the process being much less efficient in energy production than the complete aerobic process, and cells which have to depend upon it are consequently under a serious biological disadvantage.

Animals and some simple plants use up energy in moving themselves from place to place, but in the majority of plants, which are stationary, it is not quite so obvious why a continuous supply of free energy should be necessary, the greatest part of it being, in fact, taken up by internal work within the living protoplasm. For example, the formations of most of the complex substances, such as proteins, from the simpler materials which enter the cells are endothermic reactions, i.e. they only occur under the influence of energy from outside. Except where photosynthesis is going on, this energy is supplied on the spot by respiration. It will be shown in the next chapter that a mechanism exists in protoplasm, which prevents the numerous enzymes and other substances which it contains from reacting together indiscriminately. The nature of the separation set up is still uncertain, but its maintenance is sure to involve the expenditure of energy, which can only be derived in the majority of cells from respiration. The slow movements of growth which shoots make into the air against the drag of gravity, and which roots make into the soil in spite of a good deal of friction also necessitate an energy supply.

Besides the energy which is absorbed in these ways a certain amount is always being converted into heat, and although the amount produced is always very small, it becomes comparatively great during periods of rapid respiration, such as that which occurs during germination. If rapidly sprouting seeds, such as barley or peas, are shut up in a thermos flask, a rise of temperature of several degrees will soon be observable.

The Respiration of Succulents. Some plants, of which the Cactaceae and Crassulaceae are notable examples, do not carry out a complete oxidation of their sugars even in the presence of atmospheric oxygen. Respiration stops when the products of glycolysis are only partially oxidized, so that no carbon dioxide is formed, but malic acid accumulates in the tissues, and the result of the reactions may be represented by the equation

$$2C_6H_{12}O_6+3O_2 \rightarrow 3C_4H_6O_5+3H_2O$$

It is possible that the reason for the incomplete oxidation lies in the bulky nature of succulent plants, which makes the entrance of oxygen by diffusion very slow. Other bulky tissues such as apples do, however, produce carbon dioxide, so it is perhaps more likely that the difference lies in the nature of the enzymes which the tissues possess, and that succulent plants are without some of the oxidizing enzymes present in the majority of plants.

A variety of organic acids, such as citric acid and oxalic acid, are known to occur in the fungi as products of respiration. Their formation seems to be the result of numerous complex reactions, and what relation they bear to the intermediate products of normal aerobic respiration is not yet known, though oxalic acid has also been found to accumulate during respiration in some of the higher plants.

The Respiration of Fatty Seeds. In the great majority of seeds there is an abundance of reserve material in the form of fats. During germination, these substances are respired, being first converted into sugars, after which respiration probably follows the normal course. The initial transformation of fats into sugars involves an oxidation which is additional to those usual in aerobic respiration, and the total amount of oxygen absorbed is thus greater than when the hexose is derived from a polysaccharide. In this case the respiration ratio, carbon dioxide produced/oxygen absorbed, is no longer 1, but something less than 1, varying with the particular fat being respired. The equation for the respiration of triolein, for example, would be

$$C_{57}H_{104}O_6+80O_2 \rightarrow 57CO_2+52H_2O$$

and the respiratory ratio would then equal 57/80.

Respiration in Plants and Animals. In certain respects respiration is similar in both plants and animals. Oxygen is absorbed and carbon dioxide given out in both cases, and in each the principal substance acted upon is a hexose. Energy is released and utilized to maintain the organism, whether animal or plant, and the release always occurs in every living cell. In the higher animals, however, there is always an elaborate mechanism, lungs or gills, in which oxygen is taken from the air into the blood and thus distributed to all the living cells of the body. The higher plants do not possess a special absorbing apparatus, and oxygen diffuses into them wherever their surface is permeable to the gas, and slowly passes on by further diffusion to all their respiring cells. The special name of *breathing* is given to the passage of air through lungs and gills, and in biology the word is not synonymous with respiration as it is in common usage. All plants respire, but none of them breathe, the latter process being confined to the higher animals.

The resemblances between plant and animal respirations must not be overstressed, however, since at present we do not know how far they are similar in their more intricate details. An important difference exists in the fact that nitrogenous substances such as amino-acids are oxidized in animals, whereas in plants this does not occur except possibly in the last stages of starvation.

All plants can exist for fairly lengthy periods upon energy derived from anaerobic respiration, but animals quickly die if deprived of oxygen. This is probably connected with the fact that plants can exist upon a much smaller energy supply than animals, which are continually expending it in muscular action.

ALCOHOLIC FERMENTATION

The ability to break down sugars into carbon dioxide and alcohol in the absence of oxygen, which is possessed by all plants, is present in yeast (*Saccharomyces* spp.) to a very exceptional extent. Yeast is a unicellular fungus, and since it possesses no chlorophyll, it is unable to form sugars itself, and must obtain them in solution from its surroundings. Yeasts are found growing naturally, for example, upon the surface of grapes, which are sweet when ripe on account of the sugar in their juice. If the grapes are bruised, or extrude juice when overripe, alcoholic fermentation sets in; that is the sugars are broken down into alcohol with the liberation of gaseous carbon dioxide. Since this easily occurring natural process is the basis of wine making, it is not surprising that the process was discovered before the beginning of recorded history.

The biological interest of alcoholic fermentation by yeast lies in the release of energy, which occurs during the process. In this respect it is analogous to the anaerobic respiration of other plants, the material products and energy obtained from a molecule of hexose being the same in each case.

The final result of alcoholic fermentation can be expressed approximately by the equation

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 9{,}400 \text{ cals.}$$

It is known, however, that although alcohol and carbon dioxide form the great bulk of the material derived from the sugar, small quantities of other substances, such as succinic acid and glycerine also remain when the action comes to an end. In addition, when yeast is fermenting in a sugar solution, about 2 per cent. of the material is permanently withdrawn, being built up into the fabric of the organism.

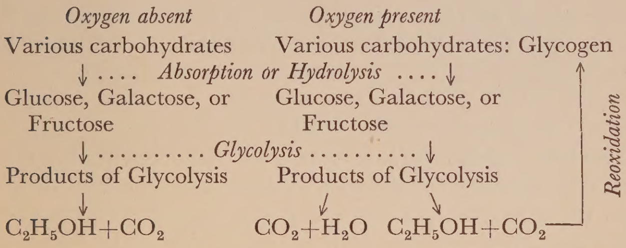
Three hexose sugars, d-glucose, d-galactose, d-fructose, are fermentable, while the other hexoses, pentoses, &c., are not. The reason for this may be that each of the first three sugars contains a common grouping within the molecule, which is attacked by the fermentation enzyme, zymase, and which is absent from the other sugars. Some yeast species will also ferment disaccharides, but the disaccharide is first hydrolysed to one or more of the fermentable hexoses.

It is not yet definitely known whether the two processes of anaerobic respiration and alcoholic fermentation are identical in the steps by which the final results are reached. Several theories have been put forward concerning the intermediate products formed during fermentation, and although some of the substances have been identified almost with certainty, the complete sequence has not yet been established.

In one respect alcoholic fermentation by yeast appears to differ distinctly from anaerobic respiration, namely in its reaction to oxygen. Yeast will continue to ferment for considerable periods in the absence of oxygen, and its protoplasm can be maintained in an active condition by the energy derived from the process much longer than anaerobic respiration will maintain the protoplasm of most plants. Nevertheless it cannot remain independent of oxygen indefinitely, and the formation of new yeast cells, which may be very rapid when oxygen is present, eventually ceases when it is absent. The effect of the gas upon this unusual metabolism is not the same as in other plants, since the fermentation products are not completely oxidized to carbon dioxide and water. A certain amount of normal aerobic respiration goes on, but this does not entirely replace the formation of alcohol as in other cases. The amount of alcohol which remains becomes much less, however, and one reason for this is that it is being reoxidized into some form of polysaccharide, probably glycogen. It has been found that when alcohol appears in ordinary plants, they are quite unable to reoxidize it, as yeast does, when brought into air. The working back of glycolysis products into carbohydrates, which has been found in some cases, occurs at an earlier stage.

It has often been suggested that alcoholic fermentation is identical with anaerobic respiration, which in its turn was regarded as an incomplete aerobic respiration. In the presence of oxygen anaerobic respiration was supposed to constitute the initial stages of the process which was completed by a final series of oxidations. As shown above, it seems from the nature of the yeast mechanism in the presence of oxygen, that the relationship between the three processes cannot be so simple as was at first supposed. An outline of the fermentation processes is set out below and should be compared with the corresponding summary of respiration on p. 104.

ALCOHOLIC FERMENTATION IN YEAST


Oxygen absent
Oxygen present
Various carbohydrates
Various carbohydrates: Glycogen
Absorption or Hydrolysis
Glucose, Galactose, or Fructose
Glucose, Galactose, or Fructose
Glycolysis
Products of Glycolysis
Products of Glycolysis
$C_2H_5OH + CO_2$
$CO_2 + H_2O$
$C_2H_5OH + CO_2$
Reoxidation

Zymase. The enzymes of yeast have been very intensively studied on account of their great practical importance in brewing, baking, and medicine. The original discovery of zymase was made during an attempt to preserve a yeast extract which had been obtained for medical purposes by grinding a mass of yeast until the majority of the cells were broken, and then pressing out the juice. Ordinary antiseptics were found to be unsuitable, and when sugar was added as a preservative, the quite unexpected result was obtained, that the non-living extract fermented the sugar to which it was added. Up to that time it had been supposed that fermentation was a property of the living cell alone, and this discovery led to the first recognition of cell catalysts or enzymes.

Without zymase, fermentation does not occur, but provided the enzyme is present it is not necessary for the yeast cells to be alive. It is not easy to separate the enzyme from cells, however, but if the latter are killed, washed with acetone, and dried, the non-living mass still contains zymase and many other enzymes in an active condition. Such a preparation as this is known as *zymin*, and when added to sugar solutions is able to cause fermentation much more vigorously than pressed juice. Unlike living yeast, it soon loses its activity, and, of course, has no powers of multiplication and regeneration. In the dry condition it will keep almost indefinitely, and is consequently a very convenient source for a large number of enzymes: as such it is used for a variety of commercial purposes, though in brewing living yeast has been found more useful.

OTHER FERMENTATIONS

The term *fermentation* is used to cover the decomposition of a number of organic substances under anaerobic conditions. In all natural fermentations, living organisms, yeasts or bacteria, are concerned, though the action is brought about not by the organism as a whole, but by specific enzymes which it contains. Alcoholic fermentation brought about by zymase in yeast cells has been the most studied of these reactions, but many others are known. Various bacteria break down the sugar in milk (lactose) to lactic acid, and so cause 'souring'; others cause the formation of the butyric acid which makes butter rancid, and the anaerobic rotting, or putrefaction, of proteins may also be included under this heading.

EXPERIMENTAL WORK

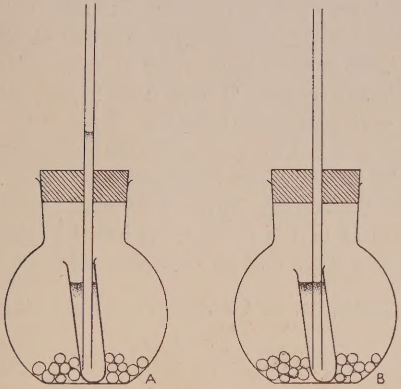


FIG. 24

Exp. 28. LIBERATION OF CARBON DIOXIDE IN AIR AND NITROGEN

Fit two small flasks (about 200 c.c.) with new rubber corks and exit tubes as shown in fig. 24. The tubes should rise at least six inches above the corks. The exit tubes are to dip into small test-tubes as shown. Fill one test-tube (*A*) with an alkaline solution of pyrogallol (freshly made up) and the other with brom-cresol purple (1 drop per c.c. of freshly boiled distilled water), and place in position. The pyrogallol solution will absorb all the oxygen in the flask *A*.

When no more bubbles are drawn in through the open tube absorption of oxygen in the flask is complete. Uncork the flasks and slip germinating peas into each with the minimum of disturbance. Recork and note the level of the liquids in the two tubes. Allow to stand in a position sheltered from draughts; considerable changes of temperature must be guarded against.

In a short time the pyrogallol will be seen to rise in the exit tube and in time may overflow. In the other flask (*B*) there will be no change of level, but the colour of the indicator will change showing the formation of acid (carbonic acid). If a little lime-water is now added to each flask and shaken round, it will turn milky, showing the presence of carbon dioxide in each.

If the flask *B* is left long enough, the indicator will begin to rise in its tube. This happens when the peas have absorbed all the oxygen in the flask and begin to respire anaerobically as in the flask *A*. A lighted splint put into the flask at this stage will be extinguished.

Exp. 29. FORMATION OF CARBON DIOXIDE FROM SUGAR BY YEAST

a. In the Absence of Oxygen. Add about 5 gm. of baker's yeast to 25 c.c. of 10 per cent. glucose solution in a boiling tube. Fit the tube with a cork and delivery tube bent into a right angle. Lead this tube into a test-tube containing lime-water. Place the tube with the yeast and sugar in a water-bath containing water at about 35° C. Bubbles of carbon dioxide will be given off, producing a milkiness in the lime-water.

After an hour uncork the boiling tube; the smell of alcohol will be noticeable.

b. In the Presence of Oxygen. Fit a boiling tube about 1 inch in diameter (fig. 25 B) with an air inlet through a U-tube (A) containing soda lime, and an exit by way of a tube

(c) containing lime-water. All the joints must be air-tight. Into the tube B put 25 c.c. of 10 per cent. glucose solution and about 5 gm. of yeast, and immerse in a water-bath at about 35° C. Connect the exit tube of C to a filter-pump, and draw a slow current of air through the apparatus, to supply the yeast with oxygen. The lime-water in C will soon become cloudy, owing to the liberation of carbon dioxide in B. If B is uncorked after an hour the smell of alcohol will be detectable.

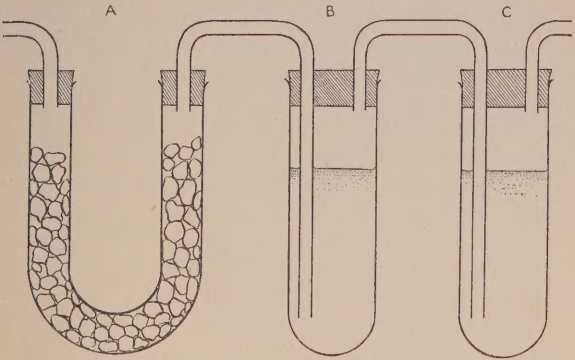


FIG. 25

Exp. 30. LIBERATION OF HEAT DURING RESPIRATION

Fill a thermos flask to about two-thirds of its capacity with germinating seeds (peas or wheat) and put in a long-stemmed thermometer, burying the bulb among the seeds. Close the neck of the flask with a tight wad of cotton-wool. Fit up a second flask with seed that has been killed by boiling. After twelve hours read the temperatures indicated by the two thermometers and continue to take readings at intervals of about 1 hour. What is the greatest difference recorded?

Large or compound flower buds such as chrysanthemum and dandelion when opening are also suitable for this experiment.

Exp. 31. LOSS OF WEIGHT DURING RESPIRATION

Count out two lots of 100 wheat grains, avoiding noticeably shrunk or damaged specimens. Weigh each lot to the nearest milligram; the two samples should not differ by more than 5 per cent.; transfer one lot to an evaporating basin, and place in an oven to dry, and after 24 hours' drying remove to a desiccator. When cool, weigh again. This gives the dry weight of 100 ungerminated seeds. Germinate the second sample on damp sawdust on a petri dish or saucer, which must be kept in the dark, and not allowed to dry up. After seven days etiolated seedlings will have formed. Remove these to an evaporating basin, after carefully washing away the sawdust adhering to the roots. This is much easier if the plants are allowed to soak in water for a while. Dry in an oven *for the same length of time as before*, allow to cool in a desiccator, and weigh. Compare the dry weight of the seedlings with that of the seeds. What percentage of the solid material has been lost? It represents the amount that has been oxidized to carbon dioxide and water, and given off during the seven days of germination; compare experiment 28, the liberation of carbon dioxide by peas.

Dried plant material is very hygroscopic and great care is necessary in cooling and weighing the samples. The time allowed in the desiccator should be the same in both cases, and weighing should be done in stoppered weighing bottles.

Exp. 32. THE RATE OF RESPIRATION. EFFECT OF STARVATION

Assemble the apparatus shown in fig. 26, and connect the tube E to a filter-pump. The exact form of the vessels A→E is not important, but those shown in the diagram reduce the number of joints, and hence the possibilities of leaks, as far as possible. If all the necessary corks and pressure tubing are of new and springy rubber, there will be no difficulty in making the apparatus air-tight; no waxing is necessary. Support each vessel individually by means of clamps and retort stands, or trouble is sure to ensue. The small gas cylinder C is to serve as the plant chamber, and must be darkened by tying it up in a black bag, or better by pasting dull black paper over its sides and base. Fill the vessels as follows.

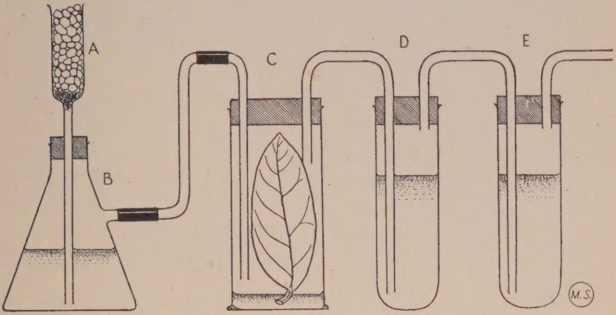


FIG. 26

A. Soda lime, which should be slightly moist.
B. About 1 inch of lime-water, which must be perfectly clear when first put into the filter-flask.
C. A freshly cut leaf of cherry laurel, about 4 inches long, the petiole dipping into water in the bottom of the cylinder.
D. 100 c.c. of N/10 baryta water.
E. Ditto.

Having filled the apparatus, turn on the filter-pump and air will be drawn through. Regulate the stream to a slow steady rate of bubbling (about 60 to the minute) in B, D, and E. Test the apparatus for leaks by corking up the open end of A for a few minutes. If the apparatus is air-tight bubbling will soon cease. Remove the cork and allow air to be drawn through the apparatus for 4 hours. Note the temperature. At the end of this time there will be a marked precipitate in the absorption tube D. If there is any precipitate in E the two lots should be run together in a larger tube or cylinder, taking care to wash in the last traces of precipitate. Cork carefully and put aside for comparison with the sample to be obtained later. A gentle stream of air must now be kept passing through the plant chamber for a week. If the tubes D and E are replaced they should be filled with pure water only. When the week has passed, refit the apparatus as before, and put 100 c.c. of N/10 baryta water into D and E again. Draw air through for 4 hours and put the final solution from D and E into a vessel similar to that containing the first sample. Compare the two; which contains the heavier precipitate, the first or the last? If there is any doubt, shake up and notice which solution appears the cloudier. The heavier precipitate indicates the greater production of carbon dioxide.

The above experiment can be made more precise by titrating the residual baryta in the absorption tubes with N/10 HCl. It will be better in this case to put 25 c.c. of baryta into each tube and make up to 100 c.c. with water free from carbon dioxide (freshly boiled distilled water, cooled under soda lime). At the end of each four-hour period pour the baryta water from the tubes D and E into a large beaker. Rinse the tubes with carbon dioxide free water, and pour the rinsings also into the beaker. Add one or two drops of phenol phthalein and titrate with N/10 HCl until the colour disappears.

100 c.c. original solution $\equiv$ 25 c.c. N/10 HCl.
Run in x c.c. N/10 HCl.

Baryta neutralized by respiratory CO_2

$$\equiv 25 - x \text{ c.c. N/10 HCl.}$$

$$\equiv (25 - x) \times 1{\cdot}12 \text{ c.c. } CO_2 \text{ at N.T.P.}$$

$$\equiv \frac{(25 - x) \times 1{\cdot}12}{4} \text{ c.c. } CO_2 \text{ per hr.}$$

V

THE PROTOPLAST

THE NATURE OF PROTOPLASM

THE previous chapters have given an account of the chemical transformations which make up plant metabolism. It has been shown how energy and material are absorbed from external sources, and together build up the fabric of the complete plant. While this is going on a large number of changes have taken place, simple enough in their general outline, but very complex in their details. All of them occur in living cells, and it is important to realize that without the intervention of such cells few of the common metabolic reactions will go on. It may be mentioned, for example, that a mixed solution of carbon dioxide and chlorophyll will not form sugars however long it is exposed to sunlight, and sugars once formed are stable substances in solutions outside the plant. They do not condense spontaneously to polysaccharides, nor do they break down to carbon dioxide and alcohol. In some cases, it is true, inorganic substances are known which will facilitate reactions when added to substances which are inert in their absence, even though they are not used up in the reaction. Such substances are called *catalysts*, and by their assistance numerous reactions which occur in cells can be simulated. Starch, for example, can be hydrolysed to glucose by boiling it with a trace of acid, though it is not appreciably changed by boiling alone. In plant cells the reverse change from hexose to starch also occurs, but this cannot be brought about artificially. There are also a great many other reactions which are known to occur inside plant cells, but which up to the present have not been brought about outside them. In other cases reactions, which are known to the chemist, occur under biological conditions in modified forms, and it becomes clear that the protoplasm, in which they are going on, exercises a controlling influence upon them.

The Visible Protoplast. It is possible to see protoplasm by examining living tissues under a high power of the compound microscope, and something of its nature can be learnt from such an inspection. The young cells that are found just behind the growing points (fig. 27 A) consist of a tiny body of protoplasm, surrounded by a very thin wall. At the centre lies a comparatively large *nucleus*, which is spherical and appears rather denser than the general mass of the protoplasm, which for distinction is called *cytoplasm*. The latter is translucent and colourless, but contains numerous minute particles which give it a granular appearance. Cells such as this are formed by the repeated divisions of the cells of the growing point (meristem). They do not remain in this condition permanently, but slowly increase their volume, while the wall around them gradually thickens. The growth of the protoplasm does not keep pace with the enlargement of the cell volume, and droplets of clear liquid appear in it at various places. On account of their empty appearance these droplets were originally called *vacuoles*, and the name has been kept, although it is known that they consist of water containing many substances in solution. As enlargement continues the vacuoles increase their size and their numbers, and finally begin to run together, and form one large drop of liquid in the middle of the cell. At this stage the protoplasm forms a sac round the single vacuole separating it from the cell wall. The nucleus remains embedded in the cytoplasm which may now also contain numerous other conspicuous bodies, collectively termed *plastids*. In assimilating cells for example, the chloroplasts are always found in the cytoplasmic lining, and never free in the vacuole.

The whole cell as thus described is a living unit, and although it is usual to recognize the protoplasm as living matter *par excellence*, it cannot be separated from the other portions of the cell and retain its properties uninjured. Vacuoles are not found in the young stages of cell growth, and cell walls are dispensed with by the cells of animal

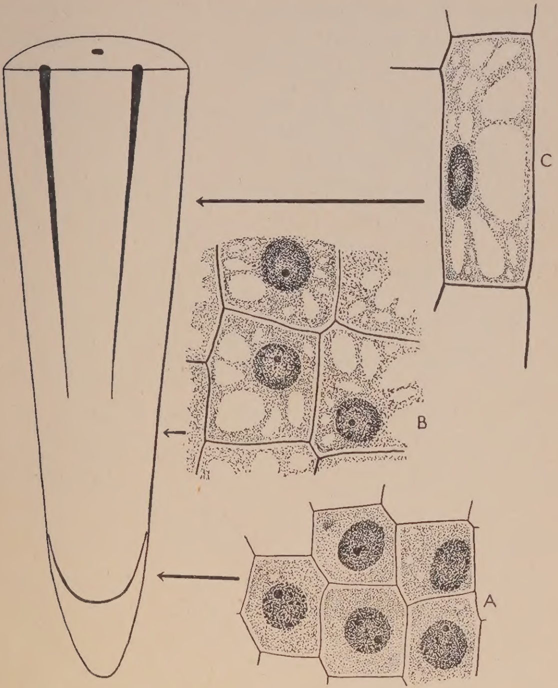


FIG. 27. YOUNG CELLS FROM THE TIP OF A BEAN ROOT
The arrows indicate the distance from the root tip of the cells in successive stages of development *A*, *B*, and *C*. The final stage of vacuolation, in which all the vacuoles have coalesced, is not shown, but occurs higher up the root. *A*, *B*, and *C* highly magnified. (M.S.)

tissues, although they are present in all plants excepting in the obscure 'slime fungi'.

When cells have been divided into two halves, one of which has contained the nucleus uninjured, while the other is enucleate, the part containing the nucleus has continued to exist and form new cells. The other half has remained alive for some time but without being able to grow, divide, or even to reform the missing cell wall. For these reasons, it seems probable that the unit of living matter is the portion of protoplasm dominated, in some way at present obscure, by a single nucleus, and this is sometimes called the *energid* or *protoplast.*

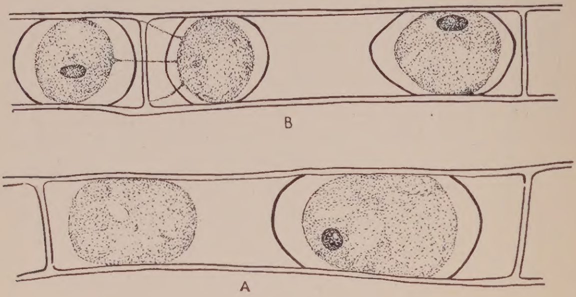


FIG. 28. CELLS FROM HAIRS OF *Cucurbita Pepo*

A shows the protoplasm separated into two parts by plasmolysis. The nucleated part has formed new walls, but not the other part
B shows protoplasm without a nucleus linked to a nucleated fragment by protoplasmic fibrils passing through the cell wall. Both fragments have formed new walls. Highly magnified
(Redrawn after Townsend)

The Association of Protoplasts. The simplest plants such as bacteria, and the unicellular algae, consist of a single protoplast living independently, but in more advanced types large numbers of protoplasts are associated together into *tissues.* The cells of a tissue are all very similar, and are usually specialized in their metabolism, so that they perform one or more processes with special efficiency. The result is that the tissue as a whole becomes a plant organ carrying on the process in question on behalf of the entire plant. Protoplasts which are grouped together into a tissue are no longer independent in their actions, and it has been shown that they are actually continuous with one another. Thin fibrils of protoplasm pass through pits in the inert cell walls, as shown in fig. 29, so that the living matter of one cell is not entirely isolated from that of the next, but is on the contrary organically connected with it.

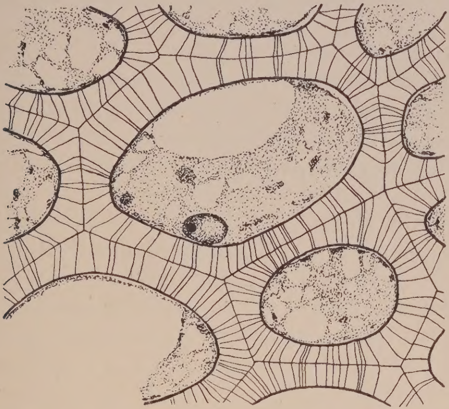


FIG. 29. CELLS FROM THE RIPE SEED OF *Diospyros ahernii* showing protoplasmic fibrils penetrating the thick cellulose walls. × about 600
(Redrawn after Quisumbing)

It is sometimes found that the protoplasm contained within a single cell wall possesses numerous nuclei instead of only one. Such a structure is called a *coenocyte* and is probably to be regarded as a collection of energids within a common wall, each nucleus dominating the cytoplasm in its immediate neighbourhood. Coenocytes are not so common as tissues composed of numerous uninucleate cells in the higher plants, but are often found among the algae and fungi. The latter have no chlorophyll, and so lack the power to synthesize carbohydrates, including cellulose, from inorganic sources. The comparative scarcity of their cell walls may be due to this inability, which they share with animals.

Protoplasmic Structure. It is at present impossible to say whether the majority of cell processes go on within the nucleus or the cytoplasm, and it is necessary to refer them simply to protoplasm, without specifying which part of it is meant. In addition to the structural details, which can be seen under the microscope, it has become clear from experimental studies, that protoplasm has a finer, or sub-microscopic, structure as well. In other words, even the clearest looking part of the cytoplasm is not really homogeneous, but consists of two or more kinds of matter, or in technical language is *heterogeneous*. A few simple considerations will make this clear. A single cell of average size occupies about a hundred thousandth of a cubic millimetre, and only a small fraction of this volume is taken up by the protoplasm. Within this constricted space an immense number of complex reactions is going on continuously, each requiring its own special catalyst to bring it about. These reactions are not all tending in a single direction at once, but are, on the contrary, very varied, as will have become clear from the preceding chapters. In spite of the smallness of the cell dimensions, it seems impossible not to regard these things as being separated from one another in some way. Certain reactions are known to be restricted to definite bodies within the protoplast; photosynthesis, for example, occurs only in chloroplasts embedded in the cytoplasm and not throughout the cytoplasm in general, while other plastids, termed *leucoplasts*, act as centres for the formation of starch from sugars in non-assimilating cells. Bodies such as chloroplasts, which are specially concerned with reductions in the cell, may be said to have a high reducing power, and it is not extravagant to think that other parts of cells may have high oxidizing powers, even although it is not possible to locate them visually. The nature of the boundary which separates a plastid from the surrounding cytoplasm is rather uncertain; still less can one define how a region of high oxidizing power is separated off from the adjoining matter. None the less it seems impossible to understand the behaviour of protoplasm without supposing the existence of such separations.

One of the most striking differences between living protoplasm and dead is the fact, that these separations break down when life ceases. This can be demonstrated very simply by grinding up any plant tissue with water, and keeping it under antiseptic conditions. Vigorous grinding ruptures and so disorganizes most of the cells of the tissue, and although many of the capabilities of the living protoplast are thus destroyed, the activity of the catalysts, or *enzymes*, which it contains is not reduced, but for a time appears to be exceptionally great. There is a general tendency towards hydrolysis, owing perhaps to the excess of water introduced into the cell materials. All the complex organic substances, polysaccharides and even proteins, tend to be hydrolysed down to simple sugars or corresponding compounds. This process, which is called *autolysis*, occurs rapidly, and when it is complete all change comes to a standstill. The simplest explanation of these facts is to suppose that the resistances set up by the organization of the living protoplasm, and hence collectively called *organization resistance*, have been broken down, allowing the enzymes free access to substances which previously were protected from them. In the end the enzymes may even interact and destroy one another.

Other methods of breaking down the internal resistances of the cell are known, notably starvation, or dosing with chloroform (*see* **exp. 43**, p. 148). Ethylene exerts a similar effect, and by applying judicious doses it has been found possible to hasten the ripening of lemons and other fruits, owing to the acceleration of the internal processes which it causes.

CRYSTALLOID AND COLLOID

The physical behaviour of protoplasm and of many of its constituent materials when separated out indicates that they exist in the *colloidal state*, and before describing the nature of protoplasm further it is necessary to explain what is meant by the colloidal state of matter. A substance in this state differs from a typical crystalloid by being unable to form a true solution, or to crystallize out from the special type of solution which it does form. The term colloid is derived from glue (colla), which when boiled with water forms a typical colloidal solution. Some substances, such as silicic acid, are able to form either a true or colloidal solution according to the method of preparation. It is not correct, therefore, to call a substance a crystalloid or colloid, since it may exhibit crystalloidal properties at one time, and colloidal at another. It may even be possible for properties of both kinds to be present simultaneously.

Those substances which do not react chemically with water behave towards it in several different ways. They may, like salt, form true solutions, in which case their molecules become separated from one another, and move freely in the water. Many of the molecules, in addition, separate into two or more groups of atoms, called *ions*, and are then said to be *dissociated.* At the other extreme lie substances like sand, which are unaffected by the water and merely sink to the bottom of it under the drag of gravity. Between the true solution and the separated mass of solid particles there lies an intermediate condition shown by substances like clay. Clay particles are much finer than sand, and when they are mixed with water only the larger and heavier sink to the bottom. The finer ones remain permanently suspended, since the resistance offered by the water is sufficient to neutralize the small gravitational drag upon them. A suspension of this kind is not a true solution because the clay particles are not single molecules or ions, but are groups of them. The name of *colloidal solution* or *sol* is used to distinguish it from a true solution, and if the suspended, or *dispersed* material is solid, or believed to be solid, since there is sometimes doubt, it is called a *suspensoid sol.* If, on the other hand, the disperse phase is believed to be a liquid, such as an oil, the name *emulsoid sol* is applied. The power of changing from the liquid sol into a more rigid *gel* condition may be restricted to the latter class, but the division between suspensoid and emulsoid is very vague. The two liquids of an emulsoid sol are never pure, but each consist of solutions more or less dilute. If one solution is made stronger, the behaviour of the colloidal solution becomes less typically emulsoid, and begins to resemble a suspensoid. Starch, for example, forms an emulsoid sol when boiled with water, and may set on cooling into a gel. The bulk of the water contains a small amount of starch in true solution, while the starch which remains dispersed in molecular aggregates takes up a certain amount of water into its particles, and to some extent resembles a liquid in its behaviour.

The particles present in a colloidal solution are generally too small to be seen even under a powerful microscope. The smallest object which can be made visible is not less than 0·2 μ (= 0·0002 mm.) in diameter, and particles of this size are still too large to remain in permanent suspension in water, but by means of the *ultramicroscope* it is possible to detect the presence of particles as small as 5 μμ (= 0·005 μ). This instrument does not make things clearly visible, but reveals them as bright specks much as dust is seen in looking across a beam of bright sunlight. If a pencil of bright light is passed through a true solution, which has been carefully freed from dust particles, the liquid when viewed at right angles to the path of the light appears perfectly clear and uniform. When a colloidal solution is similarly treated it appears milky owing to the reflection of some of the light from the surfaces of the dispersed particles. If the solution is examined in this way through a microscope, instead of with the unaided eye, the light reflected from the particles appears as separate bright specks instead of as an unresolved opalescence.

TABLE OF MAGNITUDES

Red blood corpuscle	about 7,000 μμ in diameter.
Clay suspension particles	about 3,000
Average bacillus	about 1,000
Limit of microscope	about 200
Colloidal particles (gold suspension)	2–15
Limit of ultramicroscope	5
Large molecule (starch)	5
Medium molecule (sugar)	0·7
Smallest molecule (H_2)	0·1

Brownian Movement. The particles seen by means of the ultramicroscope are in perpetual jerky movement, and this character is also shown by very small particles directly visible under a high magnification. (*See* **exp. 42.**) It is named Brownian movement after the first man to observe it, the botanist Robert Brown. More recent investigators have been able to show that the movements are caused by the bombardment of the visible particles by the molecules of the liquid. Since the movements of the molecules have no fixed direction, small bodies will be readily displaced by the unequal impacts they are always undergoing. Larger bodies exposing greater surface will be struck by greater numbers of molecules simultaneously, and these haphazard impacts will neutralize one another more or less completely. Brownian movement always occurs when a liquid such as water is present, but if a viscous liquid is employed, the movement of the solid particles is naturally much retarded, and in a jelly is completely stopped. This affords a means of examining the physical state of protoplasm, at any given time, since it always contains numerous minute granules, whatever its condition (**exp. 42 *b***).

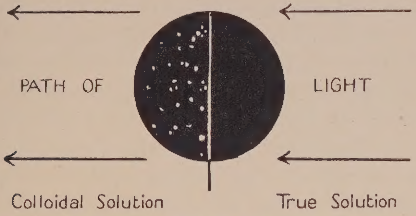


FIG. 30. Microscopic field with a beam of light passing at right angles to the tube, not into it. If the light is passing through a true solution there is complete darkness, but if through a colloidal solution the field appears as on the left-hand side of the diagram

Diffusion and Dialysis. Compared with those in true solutions, the particles in colloidal sols are large, and generally much less numerous, and as a consequence diffusion is much slower. By dividing two solutions by a thin membrane, the passage of large particles can be prevented without stopping that of finer ones. Saturated parchment paper, for example, has pores of such a size that it will allow most molecules to pass through it, but the very large molecules, or aggregates of molecules, found in colloidal solutions cannot penetrate. It was in fact this property of parchment paper which provided the first rough distinction between crystalloid and colloid solutions, and substances which could not penetrate such a membrane were said to be colloidal. There is, of course, no sharp division of the sizes in which dissolved particles can exist, but rather a fine gradation from the comparatively large, right down to the very small. Thin membranes of collodion can be prepared, having pores of various sizes according to the concentration of alcohol in which they are washed. Typical crystalloids, such as common salt, will pass through the finest pores; larger molecules, such as those of Congo Red, which give a border line solution between crystalloid and colloid, will only pass through coarser membranes, and typical colloids, such as egg albumen, will not pass at all.

This property can be utilized to separate crystalloid and colloidal substances; the mixed solution is placed in an open vessel, one end of which is closed by a parchment paper, or other suitable membrane. This is immersed in an outer jacket containing pure water. The small molecules and ions of the crystalloid diffuse slowly through the membrane into the solvent outside, but the larger colloidal particles are retained. If the outside water is repeatedly changed, or a continuous flow passed through the vessel, practically the whole of the crystalloid is eventually removed. This process is called *dialysis* (**exp. 38**), and is frequently employed in purifying colloidal substances.

Gels. It has already been mentioned that one of the characteristic properties of colloidal solutions is the ability to form gels. Concentration has an important effect in deciding whether a sol or a gel will be formed, but very little 'solid' material will suffice to form a gel. If, for example, agar-agar, a mucilage derived from seaweeds, is used, one part in a hundred of water will set it into a firm jelly, if the mixture is first heated and then allowed to cool. How this small amount of matter holds the 99 per cent. of water in a more or less rigid condition is still not clear. It may be due to the fact that in the gel condition the agar forms a sponge, holding the water in isolated droplets, and this view is rather supported by the fact that great pressures are required to squeeze out any water mechanically.

Many gels will return to the sol condition if further water is added, though the application of heat is usually necessary as well, and may be sufficient by itself (**exp. 35 b**). In these cases, the gels are said to be reversible, but in others, the *coagulation* caused by heat is irreversible, the most familiar example being the hardening of egg white (egg albumen) on boiling. There are also enzymes which are able to convert sols into gels, and vice versa, and changes of acidity may have similar effects.

Many gels are able to take up large quantities of additional water. They swell considerably in doing so, particularly if a small amount of an acid or neutral salt is present (*see* **exp. 39**). The process is called *imbibition*, and is, perhaps, osmotic, one phase of the gel acting as a membrane (*see* p. 158).

Rather contrary to what might be anticipated, it has been found that gels offer comparatively little resistance to the diffusion of small molecules. Even when the gel is a stiff one containing a fairly large amount of agar, gelatine, &c., the rate of diffusion of the molecules is not much less than it would be through pure water. This helps to explain why inorganic substances in solution pass so readily through the membranes of living cells.

Surface Action and Adsorption. The surface layers of substances possess physical properties which are not shared by the substances as a whole. One of the most important of these is the presence of *surface tension*, which causes them to behave as though they were stretched. This is most easily seen in liquids in contact with air, since they very easily run into round droplets, presenting the minimum surface possible. In order to increase the amount of surface, work must be done, as in the familiar example of blowing a bubble by forcing air inside a soap film. One may picture the surface molecules of the film as being dragged apart from one another against their tendency to cohere. The condition thus set up is very unstable and the film easily returns to the form of a small droplet in which surface area, and in consequence surface tension, are at a minimum. The more the surface is expanded, the greater is the amount of energy which will have to be present to maintain it in this condition.

When a solid block of material is broken into fragments, the pieces present much more surface than the original block. For example, a cube with 1 cm. sides has a surface area of 6 sq. cm.; if broken into pieces having sides 10 $\mu\mu$ long, approximating to the size of an average colloidal particle, the area would be multiplied a million times. The amount of surface of a solid body is thus enormously increased when it goes into colloidal solution, and since it still consists of groups of molecules its total surface energy is very great.

The actual amount of energy present per unit of surface depends upon the nature of the material. For example, mercury in contact with air has a very high surface tension, whereas oil in contact with water has a very low one, with the result that the oil easily spreads over a large area of water, instead of running into round droplets like mercury in air. The presence of substances in solution in the water may affect the surface tension to a very marked extent, in many cases lowering it considerably. The most familiar example is the action of soap in lowering the surface tension of water against air. It is a matter of universal experience that any change which causes a reduction of free energy may be expected to occur, and when a substance such as soap is added to water it tends to collect at the surface because by this means free surface energy is diminished. If there are two immiscible liquids, such as oil and water, in contact, it is immaterial which of them originally contained the dissolved substance, as it will always accumulate at the dividing surface. This local concentration is called *adsorption*, and since colloidal solutions possess an enormous amount of internal surface between the two components, adsorption of foreign matter very readily occurs, and may lead to the heaping-up of considerable amounts of material.

Adsorption is not limited to the surface between two liquids, although such cases are probably of the greatest importance in the living cell. Any finely divided solid, such as powdered charcoal or platinum black, very readily adsorbs substances from solution in a neighbouring liquid, or gases from a free atmosphere.

THE PHYSICAL STATE OF PROTOPLASM

Protoplasm behaves at most times as a stiff or viscous liquid. This is suggested by the way in which other liquids, such as the solution in the vacuoles, take on rounded forms like drops of oil in water, and in addition to this protoplasm which is free of cell walls can be filtered through glass wool, and will afterwards run together again into a single mass. The stiffness, or *viscosity*, of the liquid varies a good deal from time to time, and in extreme cases it may even set into a firm jelly, and in this condition will take up large quantities of water by imbibition. The protoplasm of resting spores is an example of this condition, but there are considerable variations of viscosity in normal vegetative cells, and a number of the properties of protoplasm seem to depend upon the ease with which it fluctuates from sol to gel, and vice versa. In the living cell these fluctuations are probably caused by changes of acidity and the action of enzymes, and if a high temperature is applied an irreversible coagulation is caused, leading to the death of the cell. It is this that causes the breakdown of photosynthesis, respiration, and other cell activities at from 40° to 50° C.

It has been noticed that when living cells are placed in a weak solution of a dye, the colour is taken up, and finally becomes much more intense in the cells than in the external solution. This is no doubt due to the fact that the dye is adsorbed upon the internal surfaces of the protoplasm, and there is good reason to believe that substances normally present in the soil solution become similarly adsorbed upon the protoplasm of root hairs and other cells.

THE CHEMICAL NATURE OF PROTOPLASM

It is impossible to separate all the non-living inclusions such as starch, fats, etc., from living cells, and then analyse the isolated living material. The processes of chemical analysis, moreover, kill the protoplasm, and may thereby cause considerable changes in it. In spite of these difficulties, it is known that protoplasm is made up very largely of complex proteins, each containing a wide range of amino-acids in its molecule. Many of these substances form colloidal solutions with water, and it is largely owing to their presence that protoplasm behaves as a colloid. They are, moreover, amphoteric (*see* p. 69), and may behave as weak acids or bases towards strong electrolytes, but in the plant conditions are such that their acid reaction is most pronounced. Potassium, for example, is always found in loose association with the proteins of plant cells, and being a strong base, may be held in a protein salt, which is easily broken down again.

ENZYMES

Enzyme Catalysis. It has already been mentioned that all plant cells contain substances called enzymes, which enable them to carry out reactions that do not occur spontaneously. These substances are distributed throughout the protoplasm, and now that we have described the colloidal nature of the matrix in which the enzymes work, we can go on to consider their own outstanding characteristics.

The simplest type of catalyst, technically called an ‘ideal catalyst’, is characterized by three properties: (1) it accelerates the velocity of one or more chemical reactions; (2) it remains itself unchanged in nature and quantity; (3) it does not alter the final result of the reaction, but only the speed at which the result is reached. If the action is reversible, each of its components is affected equally by the catalyst, so that the final equilibrium point is not altered, although it is attained more rapidly.

A mechanical example may serve to make clear the relation of the catalyst to the substances it works upon. If a heavy block is placed at the top of a smooth inclined surface, it will slowly slide to the bottom, but if the surface is well greased to reduce frictional resistance, it will arrive at the bottom much more rapidly. The action of the grease is analogous to that of a catalyst, since it does not alter the final position of the block, but only the speed with which it arrives there. The grease does not alter the amount of energy, which the block possesses in virtue of its position, but reduces a resistance, due in this case to friction, and we can imagine that catalysts similarly remove resistances to the chemical reactions in which they are concerned. The most important of the simple catalysts is the hydrogen ion, which is set free by all acids in solution.

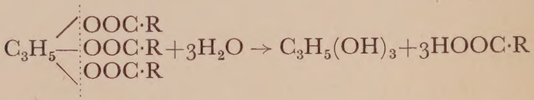
There is a special class of catalyst, whose action is due to the possession of abundant surface upon which the reacting bodies may be adsorbed or chemically combined. An example of this class is powdered charcoal, which readily adsorbs a variety of substances. It will, for example, take up 170 times its own volume of ammonia, and 78 times its own volume of carbon dioxide. If powdered charcoal is first allowed to adsorb sulphuretted hydrogen, and is then transferred to an atmosphere of oxygen, oxidation of the first gas is accelerated to such an extent that the heat set free causes the charcoal itself to ignite.

Enzymes belong to this class of catalysts, which act in virtue of their surface. They form intermediate compounds with the primary reacting substances, or *substrates*; the union is probably chemical, but in some cases the substrate may be adsorbed upon the enzyme surfaces. Enzymes differ from ideal catalysts in the following respects: Like the medium in which they act, they are themselves colloidal, and consequently are very sensitive to the influence of heat; at temperatures from 0° to 35° C., enzyme activity increases with temperature, but above 35° C. it is much retarded, due probably to heat coagulation. In the simplest cases enzymes remain, like other catalysts, unchanged at the end of the reaction. Invertase, for example, has been made to hydrolyse 200,000 times its own weight of sucrose without losing its activity, but in other cases the enzyme may lose its powers comparatively rapidly. This is due to such causes as the interaction of the enzyme with end products of the primary reaction. Finally, the activity of enzymes varies very markedly with the acidity of the medium, and every enzyme has an optimal acidity at which its activity is greatest. For example, the enzyme *pepsin*, that hydrolyses proteins to polypeptides, works best in a slightly acid medium, whereas the associated enzyme *erepsin*, that continues the hydrolysis to form amino-acids, is most effective in a somewhat *alkaline* solution, and will hardly work at all on the other side of the neutral point.

Nearly all biological reactions are reversible, and theoretically one would expect enzymes to accelerate them equally in both directions. It is readily shown outside the plant that enzymes will perform the hydrolysis of carbohydrates, fats, proteins, etc. (**exp. 33**), but it is much more difficult to show that they accelerate the corresponding condensations. This is owing to the fact that under laboratory conditions the reactions can only be performed in the presence of an excess of water, i.e. in solution. When a dehydrating agent, such as a large amount of glycerine, is added to the solution, condensation may, however, be effected, and has actually been demonstrated for a fat. It is usual to assume, therefore, that enzymes catalyse both upgrade and downgrade reactions inside the plant.

Extraction and Purification. The majority of enzymes are soluble in water, and it is, therefore, an easy matter to extract them from bruised tissues. The solvent used may be either pure water or a solution of glycerine. *Zymase* is exceptional and cannot be extracted by either of these agents, but can be got out of yeast cells by applying pressure, suggesting that it is perhaps mechanically held by the protoplasm. It is interesting to notice that a sort of natural extraction process goes on inside the plant, since many enzymes have been found in the transpiration stream. When extracted, they can usually be precipitated by adding alcohol to the solution; the precipitate can then be filtered off, and if dried at a low temperature will keep indefinitely without losing activity. Such a powder as this is not of course a pure enzyme, and the attempts which have been made at purification have so far met with only partial success, and the chemical nature of enzymes is, therefore, still uncertain. A few appear to be proteins, whereas others show a complete absence of protein reaction, and seem to consist largely of molecular groupings similar to sugars. It is not even certain that enzymes are true chemical compounds; they may on the contrary consist of a mixture of compounds, and many of them are in fact only active when auxiliary substances, called *co-enzymes*, are present. The complexity of enzyme systems is well illustrated by zymase. In addition to the enzyme proper, which is easily destroyed by heat, it also contains a co-enzyme which is unaffected by heat, and can be separated from the enzyme by dialysis. Even when these two substances are brought together, they are still unable to ferment sugars unless suitable phosphates are also added.

Specificity. It has already been pointed out (p. 112) that zymase acts only on the three sugars, d-glucose, d-fructose, and d-galactose, which possess a common grouping in the middle of their carbon chain. The enzyme works by splitting the sugar molecules at this point, and every other enzyme works in a similar manner, the action of each being restricted, that is to say, to a single characteristic grouping. Thus the enzyme *lipase* hydrolyses fats into glycerine and fatty acids,



$$C_3H_5\begin{array}{l}\diagup\,\vdots\,OOC\cdot R\\ -\,\vdots\,OOC\cdot R+3H_2O \rightarrow C_3H_5(OH)_3+3HOOC\cdot R\\ \diagdown\,\vdots\,OOC\cdot R\end{array}$$

The nature of the fat depends upon the radicle R of the fatty acid; but the enzyme operates upon groupings which are common to all fats, splitting them at the position of the dotted line, and the one enzyme is thus able to catalyse the hydrolysis or condensation of any fat. The groupings involved in the hydrolysis of a carbohydrate are quite different, and accordingly lipase has no effect upon them, and other enzymes such as diastase come into action. The adjustment of the enzyme to the molecule of the substrate it acts upon is so fine that it has been suggested that they fit like lock and key. That the space arrangement of the molecules is important is shown by the fact that the enzyme *emulsin* will hydrolyse one set of glucosides but not their stereo-isomers (*see* p. 48).

Classification. Since the chemical constitution of enzymes is still uncertain, they are classified according to the reactions which they catalyse, and from a biologist's standpoint this plan has advantages. The best-known enzymes together with their special activities are indicated below; many less familiar ones have also been identified.

1. Hydrolysing and Condensing Enzymes

Lipase		catalyses	Fats $\rightleftarrows$ fatty acids + glycerine.
Diastase	Amylase	catalyses	Starch $\rightleftarrows$ maltose.
	Maltase	,,	Maltose $\rightleftarrows$ glucose; some glucosides $\rightleftarrows$ sugars, etc.
Cytase		,,	Cellulose $\rightleftarrows$ glucose.
Invertase		,,	Sucrose $\rightleftarrows$ glucose + fructose.
Prunase		,,	Some glucosides $\rightleftarrows$ sugars, etc.
Proteases (pepsin, erepsin, etc.)			Proteins $\rightleftarrows$ various products of hydrolysis.

2. Splitting Enzymes

Zymase	catalyses	Sugars $\rightarrow$ alcohol + CO_2.

3. Oxidizing and Reducing Enzymes

Peroxidase	catalyses	Oxidations in presence of peroxides.
Oxygenase	,,	Oxidation of catechol.
Reductase	,,	Oxido-reduction.

Further details of the individual behaviour of these substances are given in the appropriate chapters.

MINERAL ELEMENTS

When plant tissues are burnt and the carbon and nitrogen driven off as gaseous oxides, there always remains behind a proportion of solid mineral matter. The quantity varies considerably, but is usually from about 2 to 4 per cent. of the total dry weight. Except in the case of encrusted tissues, which yield a great deal of calcium oxide when burnt, roughly half the mineral residue or *ash* consists of potash (K_2O). The other half is made up of various oxides, the most important being those of magnesium, calcium, and iron among the metals, and phosphorus and sulphur among the non-metals. Besides these, small quantities of other oxides are often found, varying according to the soil in which the plant grew. Thus plants from salt marshes contain comparatively large amounts of sodium which remain in the ash when the plant tissues are ignited. The following is a list of the elements which have been identified at various times in the ashes of plants:

Sulphur
Phosphorus
Chlorine
Bromine
Iodine
Fluorine
Boron
Silicon
Potassium
Sodium
Lithium
Rubidium
Magnesium
Calcium
Strontium
Barium
Zinc
Mercury
Aluminium
Thallium
Titanium
Tin
Lead
Arsenic
Selenium
Manganese
Iron
Cobalt
Nickel
Copper
Silver

The Elements Essential for Growth. Not all the elements which enter plants from the soil are necessary to their existence, and the great majority of plants grow healthily if many of the above elements are entirely withheld from them. This can be managed by growing the plants in pure sand, watered with suitable solutions. This method is called 'sand culture', but many plants can be grown merely by allowing their roots to enter the desired solution without any solid basis to ensure its aeration. In 'water cultures' of this kind many cereals will grow and ripen a good yield of grain, providing that the six elements, calcium, magnesium, potassium, iron, phosphorus, and sulphur are present, in addition to nitrogen. Together with carbon from the air and oxygen and hydrogen from soil or other water, these make up the ten elements which are necessary to the growth of all green plants. Many fungi and bacteria can dispense with calcium, but animals require iodine and sodium, which are not necessary to plants.

Mineral Elements in Protoplasm. Mineral elements do not occur in the familiar products of metabolism, yet without them its processes cannot be continued. Magnesium forms part of the chlorophyll molecule, so its importance for photosynthesis can easily be appreciated, and in some obscure way iron is also necessary for chlorophyll formation, though it does not enter into the molecule when formed. The action of the other elements is less localized, and even magnesium and iron may have further importance to the plant. All of them are found where living protoplasm is abundant, and form an essential feature of it; without them its characteristic properties disappear. Sulphur is a constituent of some plant proteins, and phosphorus occurs in the special nucleo-proteins, which are said to be localized in nuclei. As such they become part of the active protoplasm, but what makes them so important in this position is still uncertain. Some other elements seem to be a necessary part of the enzyme systems, which give protoplasm its characteristic powers. Potassium seems to be important in relation to hydrolysing and condensing enzymes; iron may be an essential ingredient in the enzymes affecting oxidations, while phosphates act as a co-enzyme to zymase. It is probable that increased knowledge will reveal still further ways in which metabolic changes depend upon the activity of these elements in the protoplasm.

EXPERIMENTAL WORK

Exp. 33. Hydrolysis by Diastase

Half fill a test-tube with a 0·5 per cent. starch solution. (For method of preparation see p. 256.) Set out a row of six watch-glasses on a strip of white paper and in each put a drop of dilute iodine solution. Dip a rod into the starch sol and transfer a drop to the first watch-glass. The characteristic colour of the starch-iodine compound will be formed. Dissolve 0·2 gm. of taka-diastase, a commercial preparation of diastase, in 100 c.c. of water and add a few drops to the starch. Shake the tube vigorously at intervals. Remove drops of the solution to successive watch-glasses after 30 seconds, 1, 2, and 5 minutes. They will then show a gradation of colour from the pure blue of the starch compound via purple to brown. The middle colour is caused by the presence of dextrin, a hydrolysis product of starch. After half an hour test again; there will be no reaction. Add some of the contents of the tube to Fehling's solution, and boil. A red precipitate will be formed, since the dextrin will have been further hydrolysed to reducing sugars (maltose and glucose).

Exp. 34. Presence of Diastase in Plants

Soak about 50 barley grains and allow them to germinate on moist filter paper for 4 or 5 days at room temperature. Then remove the sprouted grains to a mortar, just cover them with distilled water, and grind to a fine mush. Filter off the liquid, which will then contain diastase and other enzymes, since they are soluble in water. Take two test-tubes and put some starch solution and some of the filtrate into each. Boil one tube immediately and allow both to stand for about 10 minutes. Then remove a drop of the mixture from each tube to a watch-glass containing iodine solution. If necessary repeat at intervals. The unboiled mixture will gradually show the dextrin colour, while the boiled mixture will continue to show the starch unchanged. The hydrolysis in the first case is due to the diastase originally present in the barley grains. A high temperature destroys the enzyme, and so no hydrolysis was caused in the boiled mixture.

Leaves of pea (*Pisum sativum*) or clover (*Trifolium pratense*) may also be used.

Exp. 35. CRYSTALLOIDS AND COLLOIDS

a. Crystalloid. Put about ¼ inch of granulated sugar (sucrose) into the bottom of a test-tube, add one or two inches of distilled water, and shake vigorously. The sugar gradually disappears, leaving a clear colourless solution, which cannot be distinguished by the naked eye from water. The process is hastened by heating the tube gently over a flame. No change occurs on cooling.

b. Colloid. Into a second tube put a similar quantity of powdered glue and add water as before. On heating, the glue dissolves, but the resulting sol is turbid. Allow the sol to cool and notice that it sets into a firm jelly (gel). Add the same amount of water again, and warm cautiously. The gel will return to the sol condition.

Glue is of animal origin; agar-agar, which is obtained from oriental seaweeds, is also convenient for this experiment.

Exp. 36. DESTRUCTION OF COLLOIDAL STRUCTURE BY HEAT

Break an egg and collect the 'white' (egg albumen) in a beaker. Add an equal quantity of water and stir until an even sol is obtained. Pour some of the sol into a test-tube and heat. It becomes white and opaque as in a boiled egg, and does not return to its original condition on cooling. The albumen is said to be coagulated, and has lost its colloidal nature.

Exp. 37. DIFFUSION OF A CRYSTALLOID INTO A COLLOID

Dissolve 6–7 gm. of sheet gelatine in water by warming in a beaker. Add three or four drops of phenolphthalein and pour into a *warm* test-tube until the tube is full. Put on one side to cool, and when the gelatine has set invert the tube with the open end dipping into 10 per cent. caustic soda or potash solution. Clamp firmly into position. As the alkali diffuses into the gel, its progress will be shown by the colour of the indicator.

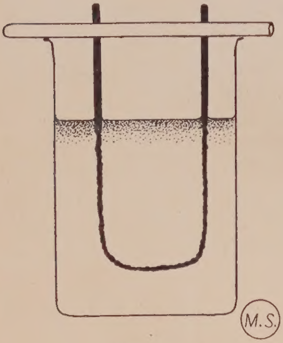


FIG. 31

Exp. 38. DIFFUSION THROUGH A PARCHMENT PAPER MEMBRANE. DIALYSIS

Make 50 c.c. of starch solution (*see* p. 256) and dissolve in it about half a gram of common salt. Pour the mixture into a parchment paper thimble (fig. 31) having a glass rod passed through its upper edges. Hang the thimble into a beaker of distilled water, which has previously been found to give no precipitate with silver nitrate. After one hour test the distilled water (*a*) for chloride (salt) with silver nitrate, (*b*) for starch by adding iodine.

Exp. 39. SWELLING OF GELATINE IN WATER

Cut a piece of sheet gelatine into a rectangle about 4 cm. by 2 cm. Place this on a piece of glass at least three or four times as large, and having a sheet of squared paper beneath it. Measure accurately the area of the gelatine rectangle, and then run on to it two or three c.c. of water from a pipette. The gelatine will at once begin to swell; when the process is complete, measure the final area. The surface of the gelatine will probably be rather wavy owing to unequal extensions in different parts, and the final area of the gelatine will, therefore, be rather greater than that read off from the flat paper. Remove the gelatine to a piece of gauze, allow it to dry and again measure its area. It will be found to have decreased again.

Exp. 40. SWELLING OF PLANT TISSUES IN WATER

a. The seaweed *Chondrus crispus* can be bought in the dried condition (carragheen). Cut a short length, say 2 cm. of the narrow lamina, and treat it like the gelatine in Exp. 39.

b. Take ten dried peas, and measure their displacement in a measuring cylinder partly filled with water. Allow them to soak in a dish for at least 24 hours, and again determine their displacement in the measuring cylinder. By what percentage has their volume increased?

Exp. 41. DEVELOPMENT OF PRESSURE DURING SWELLING

Take a small tin with a tightly fitting lid, and punch several small holes in the bottom. Pack the tin tightly with dry rice and stand in a dish of water. As the water enters from below the rice will expand and finally push up the lid.

Exp. 42. BROWNIAN MOVEMENT

a. Dilute a drop of Indian ink until the colour is scarcely perceptible, put a little on a slide and observe under the high power of a microscope. The smaller solid particles will be seen performing rapid jerky movements.

b. Keep a thin-leaved plant, such as *Elodea* or a moss, in the dark until the green colour is lost. This usually takes about a week. Then mount a single leaf and examine under the microscope. The smaller cell inclusions will be in Brownian movement, owing to the increased fluidity of the starved protoplasm.

Exp. 43. ACTION OF CHLOROFORM

Peel a piece of potato, dry the cut surfaces with blotting paper, and suspend by a piece of thread in a corked bottle. No sap will come out. Add a drop or two of chloroform and cork the bottle again. After a short time the surface of the potato will become moist, and finally sap will drip off it on to the bottom of the bottle. Chloroform makes the cell membranes more permeable, so that they no longer retain the sap as in the natural condition.

Exp. 44. ACTION OF HEAT AND WATER

Count out ten dry peas, and ten which have been soaked. Heat both lots to 50° C. in a beaker suspended in a water bath; then soak the dry peas and sow both lots in separate pans. Most of the peas which were heated when dry will germinate, but few or none of the others.

Exp. 45. PRESENCE OF MINERAL SUBSTANCES IN ASH

a. Ashing. Weigh out about 5 gm. of air-dried plant material; the species is unimportant. Transfer to a crucible or vitreosil dish and ignite, preferably in a fume chamber. Heat the residue to a dull redness (a greater

Exp. 45. PRESENCE OF MINERAL SUBSTANCES IN ASH (contd.)

heat will drive off potassium) for 3 or 4 hours. Allow to cool in a desiccator.

b. Detection of Metals. If possible a systematic analysis of the ash should be carried out. If this cannot be done, the presence of some of the important metals may be shown as follows:

(1) *Potassium.* Take up a little of the ash on a looped platinum wire, and hold in the violet flame of a bunsen burner. The pale violet flame of potassium will be easily detected.

(2) *Calcium.* Moisten the platinum wire with concentrated HCl and take up a further quantity of the ash. On entering the bunsen flame, it will now give the brick-red flame of calcium.

(3) *Iron.* Dissolve a small quantity of the ash in dilute nitric acid and filter. To a portion of the filtrate add potassium ferrocyanide. A dark-blue colour, ferric ferrocyanide, will be formed.

Magnesium cannot be satisfactorily detected without systematic analysis.

c. Detection of Non-metals.

(4) *Phosphorus.* To a further quantity of the filtrate from **b.** (3) add nitric acid and ammonium molybdate. A canary yellow precipitate of ammonium phospho-molybdate will be formed.

(5) *Sulphur.* Make a strong solution of the ash in concentrated hydrochloric acid. Decant off the liquid from the insoluble residue, dilute to about three times the volume, and filter. Divide the filtrate between two test-tubes and to one add $BaCl_2$. A white turbidity of $BaSO_4$

Exp. 45. PRESENCE OF MINERAL SUBSTANCES IN ASH (contd.)

will be formed. The amount of sulphur in plant ash is small, but by viewing the two tubes against a dark background, the formation of $BaSO_4$ will easily be seen.

Exp. 46. WATER CULTURES

Common glass bottles holding about 500 c.c. of solution and stoppered by corks having a hole about half an inch in diameter are required. Each bottle should be fitted with a covering cylinder of dark-brown paper, which can be slid on and off. Immediately before use the bottle should be thoroughly washed with boiling water, and the cork well scalded and then allowed to dry. The seedling will afterwards be fitted into the hole in the cork by a plug of clean cotton-wool, with the seed just below the cork. It is important to see that this plug and the cork are kept scrupulously dry, and away from the culture solution. If the seedling gets damp at this point it will probably damp off, becoming a prey to fungal attacks. If the cotton-wool becomes wet at any time it should be removed and a new plug put in its place.

Barley is one of the best plants to grow in water culture, and should be started not later in the year than the middle of April. Seeds should be germinated upon damp sawdust, or better upon a sheet of cork floating upon water and drilled with small holes to allow the passage of the roots. The cork should be coated with paraffin wax. When the seedlings have well-developed first leaves—after about ten days—they should be transferred to the bottles, and fixed into the corks as previously explained, the bottles having been filled with their solutions to within an inch of the corks, so that the roots alone enter the liquid (*see* frontispiece).

The following solution gives good results with barley, and should be renewed every third week. If growth is vigorous water will eventually be lost rapidly by transpiration, and distilled water should be added from time to time.

$CaSO_4.2H_2O$	0·25	gm.
$CaH_4(PO_4)_2.H_2O$	0·25	,,
$MgSO_4.7H_2O$	0·25	,,
NaCl	0·08	,,
KNO_3	0·70	,,
$FeCl_3.6H_2O$	0·005	,,

Make up to 1 litre.

For cultures lacking various elements substitute as follows:

Potassium:	replace	KNO_3	by 0·59 gm. $NaNO_3$
Calcium:			
	replace	$CaSO_4.2H_2O$	by 0·20 gm. K_2SO_4
		$CaH_4(PO_4)_2.H_2O$	by 0·71 gm. $Na_2HPO_4.12H_2O$
Iron:	omit	$FeCl_3.6H_2O$	
Nitrogen:	replace	KNO_3	by 0·52 gm. KCl
Phosphorus:	replace	$CaH_4(PO_4)_2.H_2O$	by 0·16 gm. $Ca(NO_3)_2$
Sulphur :			
	replace	$CaSO_4.2H_2O$	by 0·16 gm. $CaCl_2$
		$MgSO_4.7H_2O$	by 0·21 gm. $MgCl_2$

VI

WATER

WATER IN AIR AND SOIL

WATER is one of the most abundant and widely distributed substances in nature. Besides the great bulk of it which makes up rivers, lakes, and oceans, practically all rocks and soils are impregnated with it; and it is present as vapour in the atmosphere. The water in each of these phases is not rigidly bound up in it, but under the influence of natural forces passes from one to another. In dry weather, when there is little water vapour in the air, liquid water evaporates from any free surfaces, such as rivers, seas, and lakes, and from damp soils. If the atmosphere becomes well charged with moisture, and is then suddenly cooled, the vapour it contains is liquified again and falls as rain. An adjustment is thus set up between soil water and atmospheric water vapour, and water passes spontaneously from one to the other to maintain the balance. The higher the temperature the more water vapour it takes to saturate the air, and hence any rise of temperature tends to cause the movement of a further quantity from the soil. Similarly the displacement of damp air by a wind bringing drier air from a distance will cause further evaporation and loss of soil moisture.

Water is not lost so easily by soil as by a free water surface because there are in the former various forces tending to keep it back. The most important of these is the imbibing power of the soil colloids, which, when water is scarce, opposes great resistance to further losses. When water is abundant the resistance thus set up is only very small, so that evaporation occurs quite easily. Clay and humus are both largely colloidal, and as a result their power of retaining water against its tendency to evaporate is very high. Sand, on the contrary, consists of large particles, which do not imbibe water, although they hold a certain amount as a film on their surfaces. The surface tensions holding these films in position are nothing like so powerful as imbibition forces, and sandy soils lose water comparatively easily.

This description applies to a bare soil, such as a ploughed field, and when a covering of vegetation has grown up several complications come in. Owing to the 'blanket' which is spread over the ground, direct evaporation from the soil surface is diminished, but the total loss by the soil may be considerably increased. This happens because the plants vaporize water more rapidly than the bare ground. Their leaves provide an increased surface, and much of the sunlight which they absorb is turned to heat, both of which things tend to make evaporation faster. Plants are thus interposed between the atmosphere, which is at most times tending to draw water from the soil, and the soil itself. They are able to withdraw water from damp soils, but lose it to the atmosphere if this is dry, or, in technical language, has a *saturation deficit*.

The amount of water in the plant at any moment will clearly depend upon two factors, the amount it is gaining from the soil (*water absorption*) and the amount it is losing to the air (*transpiration*). The net gain or loss will be the difference between the two, and the total amount in the plant will be the amount by which gain has exceeded loss since the plant began to germinate. Without such an accumulation aerial plants could not exist, since water is an integral part of all their substance. The amount of water which passes through a plant from soil to air is much greater than the amount which becomes imbibed in protoplasmic colloids, or is otherwise retained in the plant. There is thus a continuous flow from the roots embedded in the ground towards the leaves spreading in the air; this is called the *transpiration stream.*

TRANSPIRATION

The forces tending to draw water from the leaves are exactly the same as those which draw it from a damp soil, but important differences between the two processes are introduced depending upon the leaf's nature. The forces opposing the loss of water from leaves are not identical with those present in soils, although they have certain similarities. For example, the colloidal protoplasm, cellulose, etc., hold water by imbibition just as do the colloids of the soil. When water is plentiful the resistance to loss thus set up is very slight, as in soils, but as water becomes scarce it increases very rapidly.

The Site of Transpiration. The structure of the leaf introduces further differences, since the cuticle is impermeable to water, and direct evaporation into the atmosphere does not occur by this path. This applies to nearly all land plants, but delicate mosses and shade plants are without such an impermeable covering. In submerged plants also there is a free passage of water across the leaf surface. By reference to fig. 2 it will be seen that the thin-walled cells of the spongy mesophyll (*b*) have a large area of contact with the intercellular spaces between them. This surface consists of thin cellulose walls, themselves saturated with water from the living cells which they contain. Evaporation goes on very readily over this area, and is often assisted by the fact that heat is being released from absorbed sunlight. The air in the intercellular spaces thus tends to become saturated with water vapour, but if the air outside is drier the vapour diffuses slowly through the stomata, and so leaves the plant.

The surprisingly large amount of gas or vapour that can diffuse through a large number of small pores, such as the stomata, has already been mentioned in connexion with the entry of carbon dioxide. The diffusion of water vapour out of the leaf is similar to the latter movement except in its direction, depending upon the fact that the higher concentration is in this case inside the leaf instead of in the external atmosphere.

In many circumstances the rate of water loss from a leaf is roughly equal to the loss from a free surface of water of equal shape and area. This may seem surprising since the stomata form only about one per cent. of the area, but it must be remembered that the actual evaporating surface, the walls of the mesophyll cells, is many times the external surface of the leaf, and that the diffusion through the stomata is not proportional to their small area.

Changes of Stomatal Opening. The stomata of most plants open by day and close at night, the period of opening and closing differing among species and to some extent according to external conditions (*see* fig. 4). When the stomata close, transpiration comes practically to a standstill, although when the air is very dry a small amount of water will penetrate the cuticle. Small changes in the size of the stomatal pores have very little effect on the rate at which water vapour passes, and it is not until closure is almost complete that the blocking effect becomes at all noticeable.

Wilting. If a leaf is transpiring water away more rapidly than it is receiving it from the stem, the bulk of water it contains naturally diminishes, and in 'soft' leaves, which depend upon turgor (*see* p. 161) for extending their laminae, such a water deficit leads to temporary drooping, which is called *wilting*. In some English mesophytes a drop of no more than one per cent. of the total water-content of the leaf causes wilting, but in other plants much greater losses are necessary.

When a leaf has wilted, the guard cells lose water and so close the stomata. Photosynthesis is brought to a standstill in consequence, and frequent wilting is thus likely to hinder the development of the plant. The drying up of the internal tissues may also cause a certain amount of injury if it is sufficiently severe, and at one time it was believed that stomata behaved in such a way as to minimize the risk of wilting, closing when water was scarce in the leaf, and opening when it was comparatively plentiful. This is not the case, however; there is actually little connexion between the opening and closing of the stomata, and the amount of water in the leaf. The guard cells respond readily to influences, principally light, which have comparatively little effect upon the rate of water loss, and are less directly affected by temperature and atmospheric dryness. Once the leaf has wilted, however, still further losses of water are prevented, so that wilting may itself retard severe drying up.

THE UPTAKE OF WATER

Water enters the plant by the root-hairs, which grow into the fine openings between the soil particles (fig. 32). They present a large area of surface, which is constantly being renewed as the lengthening roots penetrate new regions. Their thin walls and protoplasmic linings are very easily penetrated, whenever any force arises which tends to drive water through them. It has already been mentioned that there are very considerable forces holding water in and upon the soil particles, and unless there are even stronger forces urging it into the root-hairs it will not enter them; the nature of these forces is explained in the following paragraphs.

Suction Pressure. Many substances take up water, some very vigorously and others only weakly. Thus sulphuric acid will take up large quantities of the vapour from saturated air, while any dilute solution will do the same thing, though much more slowly. The cause of the absorption may be different in different cases, but it is clear that as a result of some force or another, pressure is being exerted upon the water in every instance to drive it into the absorbing material. Any pressure of this kind can be called a *suction pressure*, and may vary in degree not only according to the absorbing substance, but also according to the conditions in which it is placed; a substance when it is already half saturated will not have so high a suction pressure as when it was quite dry.

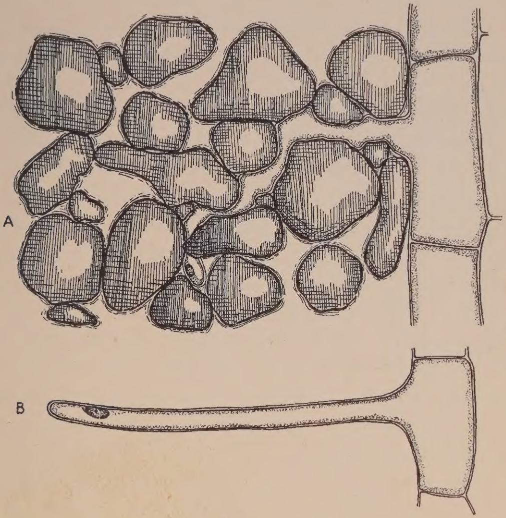


Fig. 32

A shows the relation between a root hair and soil particles, which may be saturated with water and have water films over their surfaces. Diagrammatic. *B*. a root hair grown in water, showing the nucleus, protoplasmic lining, and single continuous vacuole

If two absorbing substances are in contact, water will pass from that with the lower suction pressure to that with the higher, until a balance is established. Thus when root-hairs are in contact with soil particles, they will only take water from them if their suction pressure exceeds that of the particles. The causes which give rise to suction pressures in root-hairs, and other similar cells, are briefly described below.

Osmotic Pressure. It is possible to prepare membranes with pores so fine that they will allow water to pass through them, but not substances dissolved in the water. Membranes which behave in this way, as a kind of molecular or ionic sieve, are called *semipermeable* to indicate their special properties. Parchment paper, for example, is semipermeable to a solution of sucrose, and if made into a bag readily allows water to pass, but retains the sugar. If such a membrane is used to separate a sugar solution from pure water, the solvent is able to pass in both directions, but the solute cannot pass at all. Under these conditions a pressure is developed which drives water from outside the membrane into the solution. The stronger the solution, i.e. the more sugar molecules there are in a given volume, the greater is the pressure which arises, and the more rapidly is water driven across the membrane. As the water enters it dilutes the contained solution, and the pressure diminishes to a corresponding degree.

The appearance of a pressure when a semipermeable membrane divides solutions of different concentrations is well known and easily demonstrated (*see* **exp. 51**). It has been named *osmotic pressure*, because it results in the flow of one liquid into another (*osmosis*). The cause of the pressure is still uncertain; there are several possibilities, including the diffusion pressure of water and surface tension of the liquids at the membrane, but none of them taken individually appears sufficient to account for it.

Osmotic pressure can be counterbalanced by other pressures acting in an opposite direction. It is possible to prepare semipermeable membranes in the interstices of porous pots, and so give them rigidity. If this is done the inflowing water rises in the pot, until the head of water opposes a mechanical pressure equal and opposite to the osmotic pressure associated with the diluted solution. If a large enough weight were placed above the original solution (fig. 33) the entry of water could be prevented, and the smallest weight able to do this would exert a downward pressure equal to the osmotic pressure tending to drive water in. If the solution contained one per cent. of sucrose a weight of just under 10·5 lb. would be required for every square inch of free surface; this is equal to a column of mercury 53 cm. high, and until a comparatively high concentration was reached the weight required would increase almost proportionately to the strength of the solution.

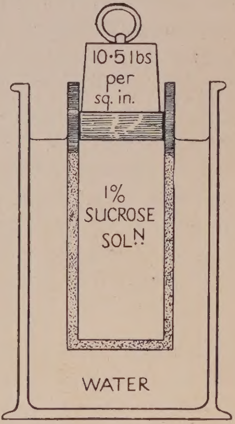


FIG. 33. THE OSMOTIC PRESSURE DEVELOPED BY A 1 PER CENT. SOLUTION OF SUCROSE

The semipermeable membrane is shaded with dots. The pressure shown would just suffice to prevent the passage of water across the membrane, i.e. to counterbalance the osmotic pressure

Wall Pressure. If the sugar solution were enclosed in a semipermeable and elastic bladder, and the bladder were then immersed in water, the entrance of the latter would first fill it, and then begin to distend it. When elastic bodies are stretched, the cohesion of their particles opposes a resistance to further stretching, and the more they are stretched the greater this resistance becomes. In the expanding bladder osmotic pressure forces water through its interstices, but the accumulating liquid sets up a hydrostatic pressure, which tends to stretch the membrane. The more liquid there is inside, the greater is the stretching, and, consequently, the greater is the elastic tension, which opposes any further entry of water. Finally the pressure thus set up becomes equal to the osmotic pressure, and there is no further movement of water, even though the concentration of sugar inside the bladder is still greater than that outside.

The suction pressure (S.P.) of the bladder at any moment thus depends upon two things, the osmotic pressure (O.P.) of its contents and the *wall pressure* (W.P.) due to the elasticity of its membrane, and the magnitude of the suction pressure is the difference between these two:

$$\text{S.P.} = \text{O.P.} - \text{W.P.}$$

If the outside solution is not pure water, but has an osmotic value of its own, this will act against the suction pressure of the cell, and reduce the absorption of water. If the two pressures are equal no water will be exchanged (*see* **exps. 53** *and* **54**). It should be noticed that the suction pressure itself changes from moment to moment as the internal osmotic pressure falls owing to dilution, and the wall pressure increases owing to increased stretching.

The Suction Pressure of Root Cells. In their relations to water root-hairs and other living cells (fig. 34) behave rather similarly to a bladder of the kind described above. The vacuole contains a mixed solution of many osmotically active substances, among which sugars are usually prominent. The protoplasmic lining is semipermeable towards many of these substances, so that an osmotic pressure is developed whenever the cells come into contact with water or other solutions. The protoplasm itself has little elastic strength, but the thin cellulose wall surrounding it provides this, though it is easily penetrated by most substances in solution (fig. 34). When the cells are fully distended they become tight and rigid like a blown-up football, and in a tissue the adjacent cells in addition press upon one another, and so the whole is made firm. Any tissue or individual cell made rigid in this way is said to be in a state of *turgor*. Many young stems and leaves owe their rigidity to this mechanism.

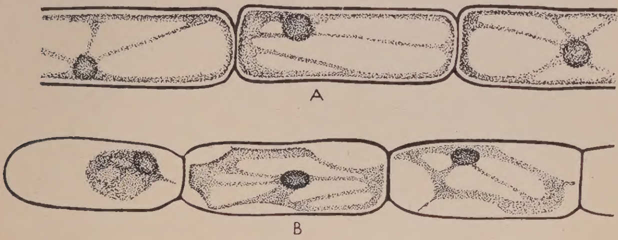


FIG. 34. CELLS FROM THE HAIRS ON THE STAMENS OF *Tradescantia virginica* showing walls, vacuoles and protoplasm, which lines the walls and also has fine threads passing through the vacuole. *A*. natural condition, *B*. plasmolysed with strong sucrose solution. Owing to the withdrawal of water from the vacuole the surrounding protoplasm has shrunk away from the walls. Highly magnified. (M.S.)

Since the suction pressure of a cell is dependent upon the osmotic pressure of its contents and the pressure developed when its wall is stretched, a root-hair can only absorb water from the soil solution if the difference between them is greater than the osmotic pressure of the soil solution and the other forces tending to hold water in the soil. The cells in the interior of roots have a great deal of dissolved matter in their vacuoles, which are consequently able to develop high osmotic pressures. When their suction pressures are greater than those of cells lying farther from the centre, they withdraw water from them, and these in their turn absorb water from others still further out. In a series of cells such as those in fig. 35 it has been found that the osmotic pressures of the contents increase in the order $a \rightarrow b \rightarrow c \rightarrow \ldots$. Thus *j* will be able to develop a higher suction pressure than *i*, and will absorb water from it until it becomes much distended, and its suction pressure reduced to that of *i* in consequence. The same relations will hold all along the series, and as long as *j* develops the highest suction pressure water will flow towards it. The suction pressures of the intermediate cells will become steady at intermediate values, and this has, in fact, been observed.

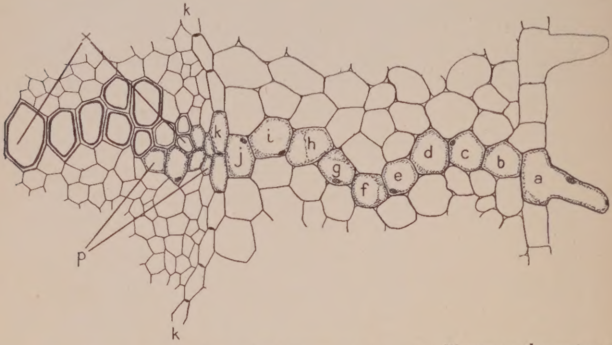


FIG. 35. Transverse section of a fragment of root to illustrate the entry of water and passage from the root hair (*a*) towards the conducting vessels (*x*) at the centre. Protoplasm shown in a single series of cells only. *b-j*. cells of the cortex; *k*. cells of the endodermis; *p*. parenchyma within the endodermis; *x*. xylem vessels. Diagrammatic. (M.S.)

THE MOVEMENT OF WATER INSIDE THE PLANT

The Passage through the Root. Water enters the root-cortex in the manner just described. The endodermis, or tube of cells, which separates the cortex from the vascular cylinder, has a special construction which limits the passage of water across it. In the older parts of roots the

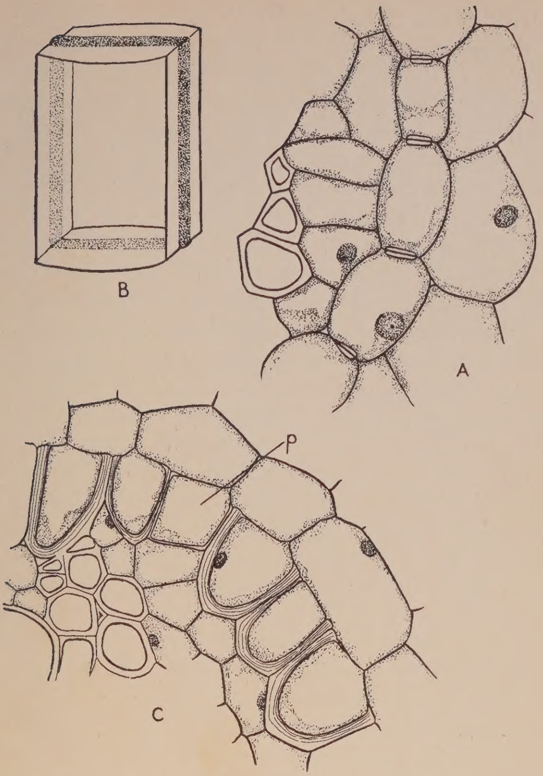


Fig. 36. ENDODERMIS OF THE ROOT

A. Part of an endodermal cylinder in the young stage showing the cutin bands on the radial walls; from the absorbing region of the root

B. diagram of a single cell to show the arrangement of the band

C. old stage, the majority of the cells having formed thick impervious deposits on their inner and radial walls. A thin walled passage cell is shown opposite the protoxylem. Highly magnified majority of the endodermal cells are quite impermeable, and the passage of water is limited to a few *passage cells* (fig. 36). In the absorbing region, i.e. where root-hairs are present, the cells of the endodermis are unthickened on their inner and outer walls, but have a band of impervious cutin running round the others (*see* fig. 36 A). The movement of water through the protoplasts is not hindered by this formation, though any passage by way of the walls themselves is prevented. Since the suction pressures of the cells inside the endodermis are lower than those outside, there is some difficulty in explaining why the water should enter them; the endodermis itself may in some unknown way be responsible. The parenchymatous cells which adjoin the dead xylem vessels pass water into their cavities under pressure, so that if a plant is cut away at the ground level, and a glass tube bound on to the stump, water is forced up the tube often to a considerable height. The pressure necessary to raise the water up the tube is supplied by the root, and is, therefore, called *root pressure*. It most probably originates in the cells which force water into the xylem vessels. How the pressure is produced, and hence the reason why water passes from the xylem parenchyma to the xylem vessels, is still a mystery, although several attempts have been made to solve it.

The Passage through the Leaf. The pressure set up in the root is not sufficient to raise the water to the tops of tall plants, especially trees, nor does there seem to be any similar action in the shoot itself, capable of supplying the deficiency. Improbable as it may seem at first sight, water is actually dragged up the stem by forces acting in the leaf, and is only slightly affected by the pushing from below.

When water evaporates from the leaf the suction pressure of the mesophyll cells rises, and this causes them to absorb water from those which are deeper seated. The movement thus started works back to the xylem vessels in the veins of the leaf, and these finally lose water to the adjacent cells.

The Tensile Strength of Water. If a penny is held horizontally in water and then lifted from it, the water follows it for a very short distance, but almost immediately runs in from the edges and drops away at the centre. If a very narrow tube, the sides of which have been thoroughly wetted, is dipped into water the liquid rises in the tube, being dragged up by its own surface tension. Once it has been raised it remains hanging from the walls of the tube, and does not run in towards the centre and finally drop back as from a flat disk. This is because the water molecules adhere to the glass and to one another, and can only be separated by the exercise of considerable force. In water, as in other liquids, the molecules will slide over one another very readily, so that the material easily changes its shape. This is quite a different matter from pulling the molecules apart, and when change of shape is

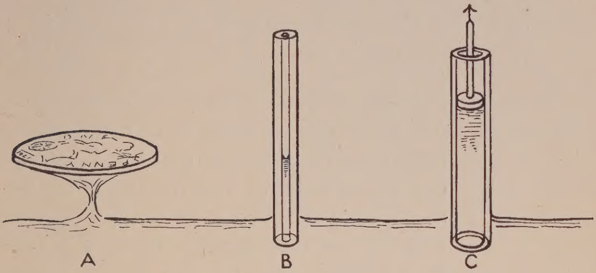


FIG. 37. THE ABILITY OF WATER TO COHERE

A. water dropping away from a metal disc raised from its surface (height of the column much exaggerated)

B. water hanging in a capillary tube

C. water being drawn up a wide tube owing to its adhesion to a thoroughly wetted surface prevented by the presence of solid walls, such as those of a glass tube, the tensile strength of the liquid becomes apparent. By suitable means much greater columns of water can be raised than those due to surface tension, and provided that there is no grease or air film to hinder contact with the glass, the water will always hang in the tube. This can be shown with a tube, such as in fig. 38, which is partly filled with water, and then boiled until all air is driven out and the sides of the tube thoroughly wetted. If it is then sealed off, the water run into the long arm, and the tube brought into the position illustrated, the water column remains hanging from the sides of the tube, even though the lower end becomes a vacuum when the steam has cooled.

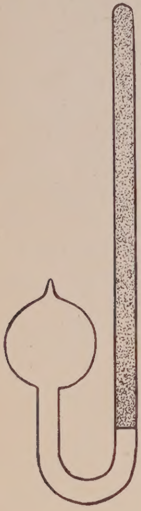


Fig. 38

The Transpiration Stream. Careful measurements have shown that in order to rupture such a column of water by a direct pull a strain of over 4,500 lb. to the square inch (300 atmospheres) is necessary, and the presence of dissolved substances does not reduce this appreciably. It follows that water columns can hang in narrow tubes for a considerable height without rupturing, and if suitable means are employed may even be dragged up them. This is what happens to the water, hanging in the vessels between the mesophyll cells in the leaf, and the parenchymatous cells of the root; it is dragged up against gravity and the resistance offered by the walls of the narrow vessels. The tension in water moving up stems in this way has been measured and does not exceed 300 lb. to the square inch, a very small fraction of the strain which the water columns are capable of bearing without rupture. The energy which initiates the movement is the latent heat of vaporization of water, absorbed during evaporation from the mesophyll cells. As already described this causes an increase of suction pressure, which works back to the cells adjoining the xylem vessels. Water is then drawn in from the vessels, and instead of breaking free from that which lies behind, the absorbed molecules drag the water columns up behind them.

The Path of Conduction. In small plants and woody twigs it can easily be shown that water passes up the stem through the xylem (**exp. 55**). In the trunks and larger branches of trees the greater part of the structure consists of woody tissue, but not all of this carries water at any one time. The central, or *heart-wood*, which usually becomes dark owing to the slow accumulation of stains, has its cavities filled with air, although when first formed water passed through its vessels. Sooner or later, and particularly in autumn, the water columns break, and once they are severed the airgap becomes larger and larger, never closing up again. The loss of conducting material is made good in the spring by the formation of new xylem elements outside the old (fig. 39), and water passes through these until they are replaced in their turn. The comparatively narrow band of water-conducting tissue is called *sap-wood.*

Even in angiosperms the xylem does not consist exclusively of vessels, but often contains in addition many tracheids, and in many woody plants, such as the pine-trees, the xylem is composed of tracheids entirely, so that it might be thought that their many cross walls would oppose insuperable obstacles to the dragging up of water. This is not the case, however, principally because the tracheidal walls are perforated by many pits, where there is only the very thin and very easily penetrated middle lamella between the two cavities. The pits linking the tracheids are of the bordered type, and the middle lamella, having a thickened *torus* in the case of *Pinus*, acts as a sort of valve. In fig. 40 the unshaded tracheid at the centre has formed a bubble of air, which has stretched until it has filled the whole cavity of the cell. The water in the surrounding tracheids is part of the transpiration stream, and hence under tension. The pit membranes are thoroughly wet, and when the water retreats they cling to its surface, and are dragged to one side of the pit, thus closing its opening. In this way the air-bubble becomes enclosed within thickened walls, which it does not penetrate at all easily, and the flow of water continues all round the blocked tracheid.

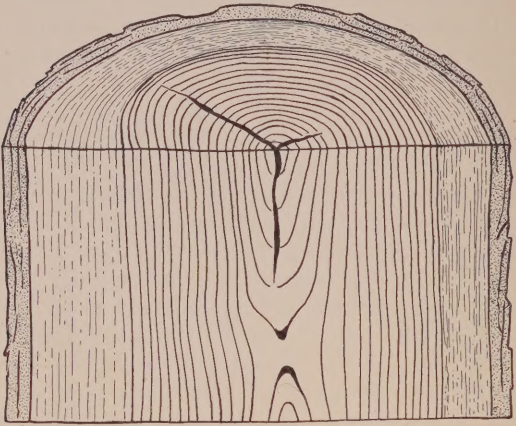


FIG. 39. A PIECE OF BLACKTHORN STEM SHOWING THE HEART WOOD AND SAP WOOD

The annual rings of the heart wood are indicated by continuous lines; those of the spring wood by broken lines; bark is shaded with dots

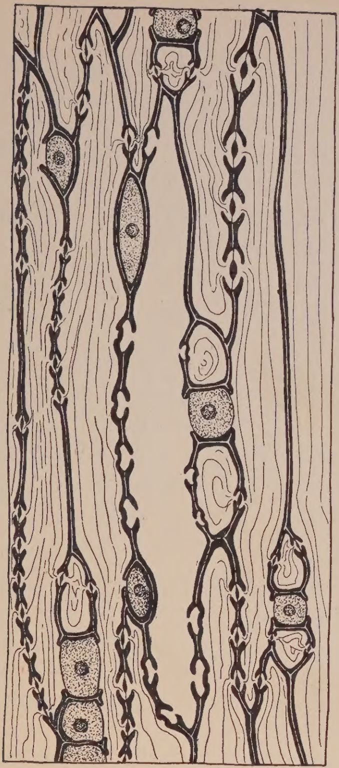


FIG. 40. LONGITUDINAL SECTION OF WOOD OF PINUS illustrating the passage of water from one tracheid to another. The direction of the flow is indicated by thin lines. The central tracheid without such lines is supposed to be occupied by air only. The membranes of the bordered pits adhere to the water surface and are dragged aside by it. When there is water on both sides the membranes lie freely in the centres of the pits. Diagrammatic.

XEROPHYTES

Plants differ very much in the type of soil and climate, which they can inhabit, and the nature of the water supply is one of the most important factors influencing their distribution. Those plants which can grow in particularly dry situations are called *xerophytes*, and as might be expected they possess certain characters, which enable them to endure such special habitats.

Drought Evaders. Many dry regions, such as the semi-deserts of Russia and America, have a short rainy period followed by a long time of complete drought. As soon as the ground is moistened by the rains large numbers of quickly growing plants appear. Their life is very short, however; they do not grow to a large size, and before the ground has dried up they have formed seed and died down again. The dry seed lies inactive in the ground until the next rainy season, so that plants of this type are not strictly xerophytes, but merely drought evaders.

Succulents. Those plants which are perennial, and hence remain above ground during the long drought, are of two kinds, and may be called *succulents* and *drought endurers*. Succulents contain large quantities of mucilages, slimy substances which are able to retain large amounts of water by imbibition, and as a consequence succulent plants do not lose the water they have absorbed as easily as normal plants, or *mesophytes*, lose theirs. The transpiration rate of an *Opuntia*, a typical succulent, was found to be about one thirtieth of that of a normal mesophyte. The water losses of succulents are often still further reduced in comparison with mesophytes by the smaller amount of surface, which their massive bodies possess, leaves and other appendages being reduced to a minimum. Their actual absorption of water is not more efficient than that of mesophytes;

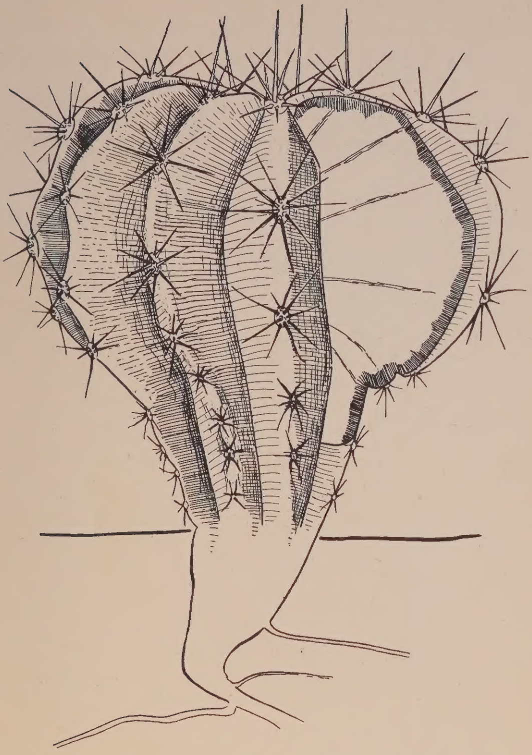


FIG. 41. *Echinocactus*, A DESERT SUCCULENT

General view of a young plant with a segment cut away on the right-hand side. The dark shading represents the green assimilating tissue. The centre is occupied by a compact colourless tissue penetrated by the vascular bundles which run towards the groups of spines situated on the ridges. Somewhat reduced contrary to what might be expected, they are usually shallow rooted (fig. 42), and when the dry season sets in uptake of water comes to a stop and their existence depends upon the slowness with which they lose the water they already contain. To illustrate this a giant cactus was kept in a laboratory without once being watered, and after six years it had only lost one-third of the moisture it originally contained. Plants of this type are characteristic of the semi-deserts of America, but are not found in their driest parts, or in the Sahara, where there are fewer short rainy periods.

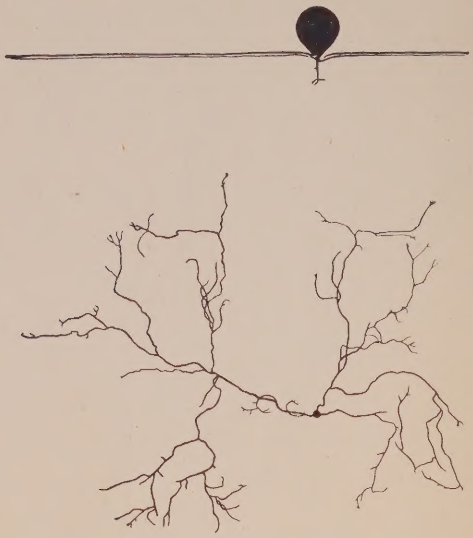


Fig. 42. ROOT SYSTEM OF *Echinocactus Wislizeni*

Vertical extension above, horizontal extension below.
(After Cannon, modified)

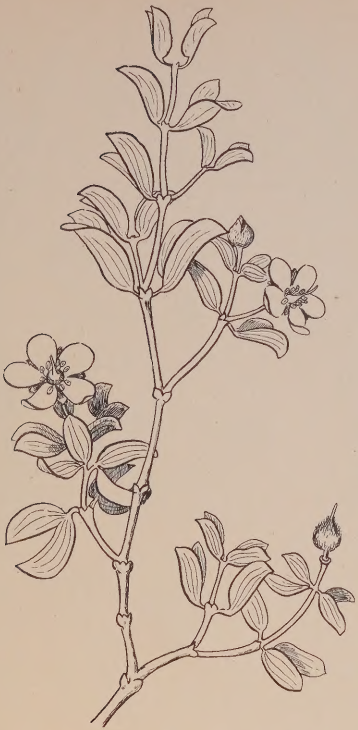


FIG. 43. SHOOT OF *Covillea glutinosa*
An extreme xerophyte, about natural size. The plant is an evergreen shrub with low much branched stems and yellow flowers. It is called the 'creosote bush' on account of the scent of its resin. (After Gray)

Drought Endurers. Drought endurers are quite different in their appearance (fig. 43), but are distinguished still more sharply by their rate of transpiration, which is usually very high. Their leaves often possess protected stomata

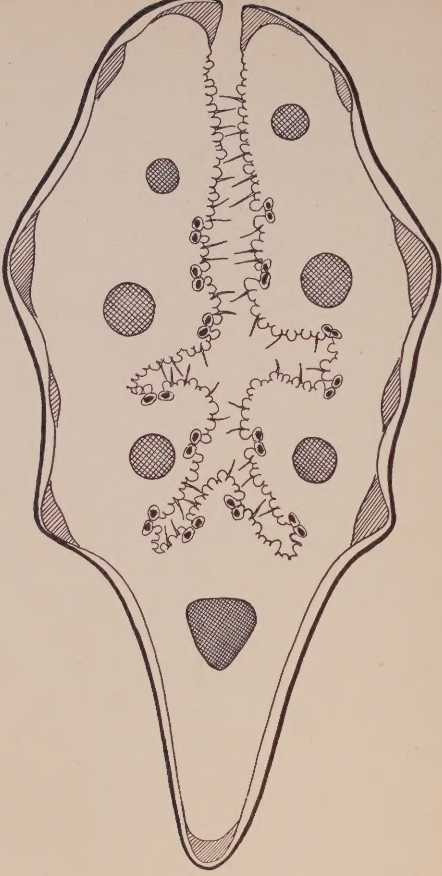


FIG. 44. SECTION ACROSS THE LEAF OF *Festuca*
in the position assumed in a dry atmosphere. The stomata, shown by pairs of black dots enclosed in circles, are in the upper surface and become enclosed in a long narrow cavity when the leaf folds. In damp air the leaf opens out flat, and the stomata are then exposed. Vascular bundles cross hatched; fibres in single line shading. (M.S.)

and thickened cuticles (fig. 44 and 45), which at first sight suggests that their transpiration rate is likely to be slower than that of mesophytes. Actually such modifications are not of much importance during normal transpiration, when the stomata are fully open, but after wilting has occurred they may be of great effect in stopping still further losses of water and complete drying up.

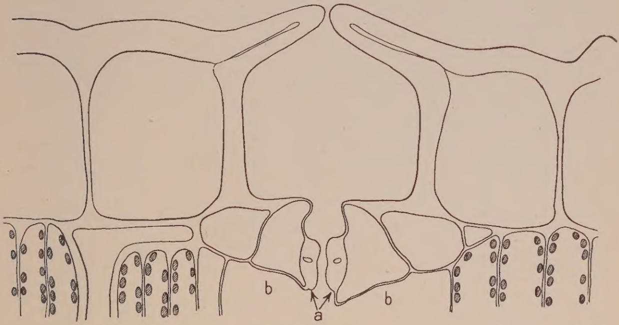


FIG. 45. STOMA AND SURROUNDING CELLS FROM A LEAF OF *Hakea*
a. guard cells; *b*. supporting cells. Chloroplasts shown in the assimilating cells. Note also the thick cuticle of the epidermis and its folds overlapping the stoma. Highly magnified. (M.S.)

If the soil is porous, plants of this type send down roots to great depths (fig. 46), and absorb the subsoil water, raising it by their unusually powerful transpiration. During the digging of the Suez Canal roots of Acacias were found at a depth of 40 feet, but desert soils are often too hard for roots to penetrate far, and then very few plants are able to survive at all. A notable exception is the 'creosote bush' (*Covillea glutinosa*) of North American deserts, which during the long droughts dries up almost completely, the merest traces of water remaining in the protoplasmic colloids. Most plants are unable to recover from desiccation such as this, but *Covillea* has the power, and it is to this special property of its protoplasm, rather than to structural modifications, that it owes its survival under such conditions. This is probably true of all those xerophytes which are really drought enduring.

There are thus several kinds of plants able to exist in dry places, and they can be classified according to the way in which they fit the conditions as follows:

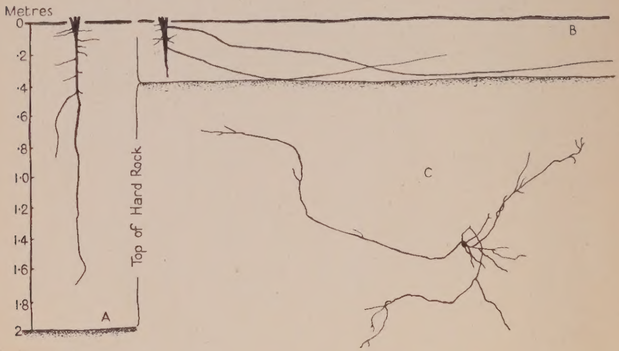


FIG. 46. ROOT SYSTEMS OF *Covillea glutinosa*

A. plant growing in deep soil; *B.* plant growing in shallow soil; *C.* horizontal extension of plant growing in shallow soil, scale rather smaller than *A* and *B.* (After Cannon, modified)

Class.	*Special Characteristic.*
1. Drought Evaders	Pass through drought as seeds; short growing period.
2. Succulents	Slow water loss in transpiration.
3. Drought Endurers:	
a. Deep rooted	Rapid transpiration; water raised from great depths.
b. Shallow rooted	Protoplasm able to recover from almost complete drying.

TRANSLOCATION

Many of the simpler substances, such as sugars, amino acids, and inorganic materials entering from the soil, are able to move about inside the fabric of complex plants. Apart from this property it would be impossible for such elaborate structures to develop, since the necessary substances would not come together and react. Movements of this sort are all included under the name *translocation*, whatever their direction or the nature of the moving substance.

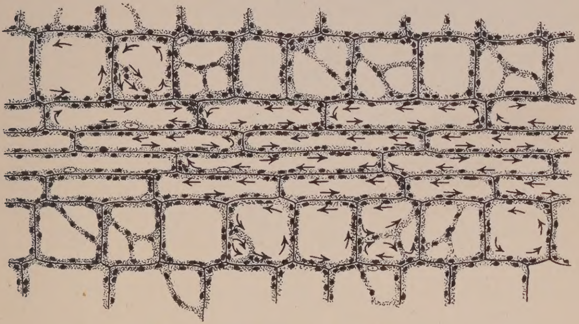


FIG. 47. CIRCULATION OF PROTOPLASM

'Midrib' and adjoining cells from a leaf of *Elodea canadensis*. Chloroplasts, shown in full black, and the nuclei where visible were carried along in the protoplasm. The arrows show the direction of movement while the drawing was being made. Highly magnified. (M.S.)

The Penetration of Internal Membranes. It is an interesting problem whether the travelling materials move themselves, by diffusion for example, or whether they are moved by forces arising only inside plants. The latter would be the case if they were carried along passively in the transpiration stream, or assisted by the circulation of the protoplasm, such as is known to occur in some cells (fig. 47),

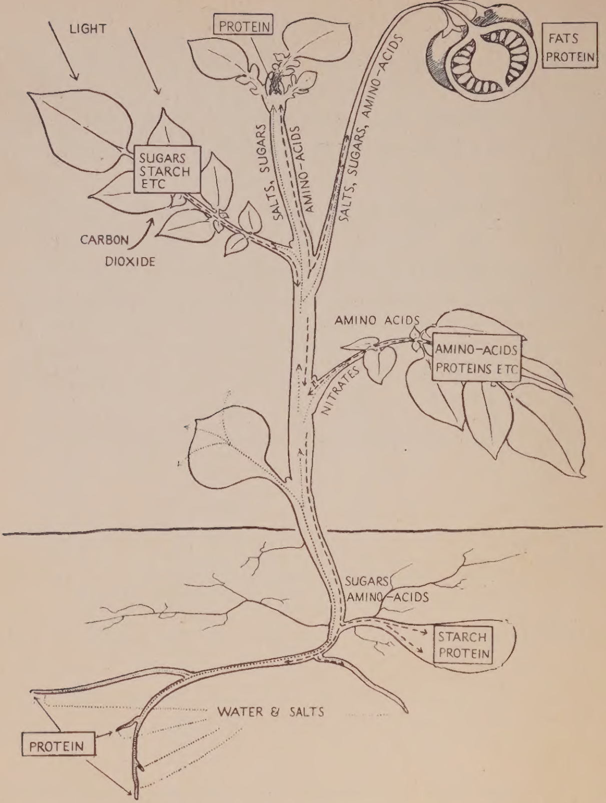

LIGHT
PROTEIN
FATS
PROTEIN
SALTS, SUGARS, AMINO-ACIDS
SUGARS
STARCH
ETC
SALTS, SUGARS
AMINO-ACIDS
CARBON
DIOXIDE
AMINO ACIDS
AMINO-ACIDS
PROTEINS ETC
NITRATES
SUGARS
AMINO-ACIDS
STARCH
PROTEIN
WATER & SALTS
PROTEIN

FIG. 48. Diagram of a plant to show the sites of the principal syntheses and paths of translocation during the period of active growth. Substances synthesized are shown in rectangles; the broken lines represent the principal routes of amino-acid and sugar movements, and the dotted lines water and salt movements. Synthetic condensations are shown going on in leaves, storage tuber, seeds and growing points; uptake of water and salts occurs in the roots

and may occur in sieve-tubes. Before attempting to answer this question it is important to state the characters which distinguish substances able to travel in the interior of plants from those which do not possess the power. The former include sugars, amino-acids, other organic acids and many inorganic ions, and probably a few of the simpler proteins. The latter are typified by polysaccharides, such as starch and cellulose, and the majority of proteins, including those conjugated with other substances. Two important properties separate these classes; their solubility in water and the size of their molecules. The mobile substances are all much more soluble than the others, and as a class they have the smaller molecules and ions; both these properties are of great importance in translocation. Solid substances can only penetrate thin membranes in solution and large ions meet with much more resistance than small ones in passing through membranes, such as cell walls and protoplasts. Soluble substances entering the root from the soil must pass through a large number of such membranes, and only those which are able to get through the wall and protoplasm of the root-hairs will effect an entrance at all. Any substance present in the soil, and fulfilling the two requirements just described, gets into the plants growing upon it, whether it does anything inside or not, and finally becomes distributed about the plant organs. That is why such unexpected elements as lead, silver, and other rarities are occasionally found in plant ashes. Similarly any soluble substance not very complex in its composition, which may be formed in leaves or elsewhere in the plant, is likely to be spread by degrees into every other part of it; translocation is not limited to those substances which may be supposed to be advantageous to the existence of the plant.

The Direction of Translocation. While it is true that substances thus spread in all directions, there are certain main streams of movement which may be recognized (fig. 48). Thus sugars tend to spread away from leaves into the stems and thence towards root and stem apices, and particularly towards ripening seeds or 'storage organs'. When a seed germinates, or a tuber sprouts, there is a further movement of sugars, together with other substances, from the storage organs where they have accumulated into the growing tissues of the seedling or sprout. Inorganic substances entering at the root spread themselves into leaves, growing points, and seeds. The movement of nitrogenous substances, nitrates, amino-acids,. and simple proteins, is not so easy to define, because their interactions and formations are not so easy to locate. Nitrates behave to some extent like other inorganic materials, and amino-acids seem to move towards the growing points, where they are condensed to proteins. The general rule seems to be that substances move from a position where they are abundant, such as the point at which they are formed or enter the plant, towards those where they are scarce, the scarcity usually resulting from their conversion into other compounds.

The direction of movement is not constant throughout the life of the plant. Thus potassium ions are travelling from the stem into the leaves all the time the latter are developing, but after their active growth has ceased a movement starts in the opposite direction, and may even result in some of them passing out of the plant and back into the soil. Many other mineral ions seem to behave similarly.

Diffusion as a Translocation Agent. Solid substances dissolved in water tend to even out their concentration by diffusion, and this may go on even across thin membranes. There is thus a resemblance between the diffusion of solids and their movements in plants, so the spontaneous movement of the molecules may be an important agent in translocation. On the other hand, it is quite clear that it is not the only influence involved, because recent measurements have shown that the translocation of sugars in stems may be more than ten thousand times faster than their rate of diffusion in water. Hormones (*see* p. 201) may also travel at surprisingly fast rates in the plant.

The Transpiration Stream. During rapid transpiration in the leaves water is carried up through the xlyem at rates much greater than those of diffusion, and the soluble substances which find their way into the stream are carried up with it. They have been found to include such organic materials as sugars and enzymes, besides the simpler inorganic ions. This rapid movement may obviously influence their distribution in the plant very considerably, and it has been claimed that side currents of the transpiration stream work in a downward direction, thus aiding transport from above.

Translocation through the Phloem. For many years there has been a notion that organic materials travelled along the phloem strands, principally in the sieve-tubes. This was mainly based on the detection of sugars and proteins in the sieve-tubes by microchemical tests, and the fact that when the phloem was cut away or killed, leaving the wood intact, translocation of these substances stopped. The evidence was not satisfactory, because the microchemical tests could not show actual movements, and in killing the phloem there was always the risk that some injury had been done to the xylem, or that decomposition products of the dead phloem injured or blocked it. The latter difficulty has never been completely overcome, but continuous careful experiments have reduced the uncertainty to smaller and smaller proportions, so that it is now difficult to disbelieve that the phloem forms the normal path of sugar and protein translocation.

The mechanism of the movement still remains obscure because of its rapidity. It has been suggested that the protoplasm lining the sieve-tubes is in constant circulation, and carries any included substances with it down the length of the tube. If the next tube has less of the included material diffusion will occur across the intervening wall, and since the distance is so short will not take very long. It has to be admitted, however, that it is doubtful whether circulation of the protoplasm occurs in mature sieve-tubes, although it has been seen in young ones and in other living cells. The agency which causes the translocation is thus still a mystery, though the tissue in which it works seems to be known.

THE ENTRY OF SOIL SUBSTANCES

The passage of dissolved substances from the soil solution into the root-hairs is in the main similar to their passage from cell to cell inside the plant. There are, however, certain differences which need to be considered, and the most striking is the fact that in this case movement often occurs, or appears to occur, from a low concentration to a higher. The total amount of dissolved substances in the soil solution is always very small, rarely rising above 2 per cent., and usually remaining much lower. After heavy rain the concentration drops considerably, and there may be no more than 0·05 gm. of solid in a hundred of water. Some of the substances vital to the existence of plants are very insoluble, and phosphates, for example, are only present to the extent of two parts in a million, yet the majority of plants absorb comparatively large amounts during their growing periods. It has been calculated, for example, that during a single growing season a crop of potatoes will remove from the soil about thirty kilograms of phosphate per hectare, and over a hundred kilograms of potash.

There are two causes which help to bring about this surprisingly large absorption. In the first place the continual passage of transpiration water into the plant must set up a current towards the root-hairs in the surrounding soil. The various substances in solution are thus brought to the absorbing surfaces at a faster rate than if there were no such current, when their replacement would depend upon diffusion alone. Once they have reached the root-hairs, however, they cannot be carried passively any farther, since the membranes of the cells behave as extremely fine sieves allowing the water to pass through readily, but retarding the dissolved substances.

The second cause of the fast uptake depends upon the fate of the materials once they have entered the plant. They may be cleared away from the first cells they enter by passing into others, where they may react chemically and so become changed into entirely new substances. In this case the rate of entry is not retarded by the accumulation of comparatively large quantities inside. Nevertheless, it remains true that even when the root cells already contain a great deal of mineral matter, still further quantities continue to enter from the soil solution which contains so little. The explanation seems to be that much of the mineral matter inside the cells is adsorbed upon their colloids, and is thus removed from solution in the cell water. If this is so the amount actually in solution may be low enough to enable diffusion to bring about the passage through the external membranes. It has also been found that under the conditions that arise in plant cells there may be a movement against the concentration gradient, owing to the electrical attraction between diffusible and indiffusible ions on opposite sides of a membrane.

Selection during Absorption. The relative amounts of the minerals found in plant ash are by no means the same as their relative quantities in the soil solution. It is clear, therefore, that different substances must be absorbed to different extents, or in other words that some 'selecting' mechanism exists. This does not imply consciousness on the part of the plant, but depends on certain physical properties of its structure and of the absorbed substances themselves. Thus, those which are readily soluble and have small ions will enter most easily, especially if they are converted to other substances inside.

At the extreme dilutions which are found in soil solutions dissolved substances exist almost entirely as dissociated ions, and it is in this form that they pass into the root-hairs. The two ions of a salt are not necessarily taken in to the same extent; thus from a solution of calcium chloride, pea (*Pisum sativum*) roots absorbed calcium ions five times as fast as chlorine ions, but runner beans (*Phaseolus multiflorus*) took up chlorine rather faster than calcium. Such a separation of dissociated ions can only happen if others are supplied to keep the balance between positive and negative charges, and in the pea experiment it was found that magnesium and potassium ions had passed out of the roots to become associated with the chlorine left behind. Sometimes hydrogen or hydroxyl (OH) ions take the place of those absorbed, and then the external solution becomes markedly acid or alkaline. In a culture solution which contains sodium nitrate ($NaNO_3$), the nitrate ions are absorbed much more rapidly than the sodium, and are replaced by hydroxyl, so that the solution soon becomes alkaline. If the nitrate is replaced by an ammonium salt the reverse effect is observed, the solution becoming acid.

The exchange of ions across membranes may accelerate their passage very much, not only in passing from soil to root-hair, but also in passing from cell to cell inside the plant. It has been found, for example, that when willow twigs sprout with their cut ends dipping into water with a little common salt in it, the chlorine ions travel into their tips much faster than the sodium ions, becoming associated with potassium in the young cells.

Physiological Balance. If a young seedling is grown in pure water its development soon comes to an end owing to starvation of the necessary mineral elements, but if it is supplied with a single salt, such as potassium sulphate, it dies even sooner. Neither potassium nor sulphate ions would ordinarily be considered poisonous to the plant, and both are, in fact, essential to its growth. The harmful effect can be much reduced by adding a second salt, especially if it contains a divalent metal, such as calcium or magnesium, but the best growth is only to be secured if all the essential elements are present in suitable proportions, or in other words are physiologically balanced. The proportions can usually be varied within moderate limits without causing much alteration in growth, but any large increment of a single substance, without corresponding increases of the others is sure to have a harmful effect. In making culture solutions for plants, it has, in fact, been found more important to balance the different constituents correctly than to fix on any absolute amount for each.

Antagonism. This name has been used to denote the property possessed by many substances of reducing the harmful effect of others, such as the action of calcium upon potassium already described. The action of strong poisons may also be reduced by the addition of certain salts to their solutions. Antagonism frequently exists between elements which do not interact chemically, so it cannot be due to the formation of harmless compounds. In some cases it may be due to the competition between the two ions for the adsorbing surfaces inside the plant cells, the second ion preventing complete blocking of the surfaces by the first. This is not always the case, however, and very often the second ion seems to prevent the other from entering the cells at all, but the theories which have been invented to account for this are too complicated and uncertain to be entered upon.

EXPERIMENTAL WORK

Exp. 47. Water Loss Occurs through the Stomata

Cut two leaves of equal size having thick cuticles and stomata on the lower surface only. Vaseline the upper surface of one and the lower surface of the other; close the cut end of the petiole by means of rubber tubing and a piece of glass rod, and then weigh each to the nearest centigram. The leaves should be suspended in such a way that no vaseline is wiped off on to the balance. Weigh again next day, when the leaf with its lower surface free will show a reduced weight owing to loss of water, and if kept for some time will become completely shrivelled, while the other is still fresh looking.

India rubber (*Ficus elastica*) leaves are very suitable for this experiment; also those of cherry laurel.

Exp. 48. Cobalt Chloride Method of Measuring the Water Evaporated. Effect of Stomata

Lay a strip of blue cobalt chloride paper (p. 255) on a small sheet of glass. Put a leaf, large enough to overlap the paper and having stomata on one side only, on top. Make a joint round the edge of the leaf with plasticine; lay a second strip of cobalt chloride paper inside the plasticine ring, and press another piece of glass down firmly on top. The cobalt chloride strips should then be enclosed in separate chambers on either side of the leaf. The emission of water vapour is indicated by the cobalt chloride paper turning pink. On which side of the leaf does this occur fastest?

The leaves of most deciduous trees are suitable for this purpose.

Exp. 49. THE POTOMETER

Assemble a potometer of the type shown in fig. 49. The container A should be a bottle of 200–300 c.c. capacity able to stand firmly, fitted with a rubber cork, with two holes. The tube B should be of narrow bore and calibrated in tenths of a c.c. Suitable measuring pipettes can be obtained ready graduated.

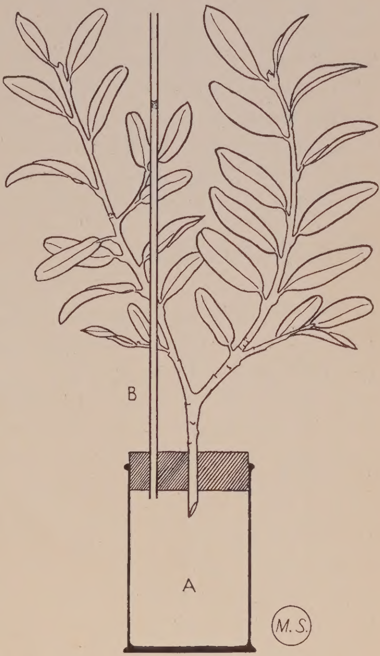


FIG. 49

a. Uptake of Water by the Shoot. Fit a woody shoot with 20–30 leaves and which has been standing in water overnight into the second hole in the cork; fill the bottle to the brim and press the cork in tightly, avoiding any air bubbles in A. The excess water will rise in the tube B; if this is not sufficient, run further water down the inner side of the tube from a pipette. Note the time taken for a definite loss of water as shown by the drop in the measuring tube; confirm the first value. This shows the rate at which water is being taken into the shoot from the apparatus.

Exp. 49. THE POTOMETER (contd.)

b. Loss of Water from the Shoot. Stand the above apparatus on a good pair of scales, sensitive to about half a gram. Weigh it and leave it to stand for half an hour or longer with the weights still on the pan. At the end of the period the scales will no longer balance owing to the loss of water from the shoot. How much has been lost?

In many cases the simultaneous loss and gain of water by the shoot are equal, but by no means invariably. After a little practice, readings of water absorption may be taken while the apparatus is standing on the balance, and simultaneous values for absorption and loss thus obtained.

Exp. 50. RAISING OF WATER BY TRANSPIRATION AND EVAPORATION

a. Suction Due to Transpiration. Cut a twig of cherry laurel, lilac, or other woody plant, such that the stump is 6–9 mm. in diameter, and 3–4 cm. long below the bottom leaf. It should have about two dozen leaves. Vaseline the sides of the stump, taking care not to block the cut end, and push it immediately into a piece of pressure tubing (fig. 50 A) attached to a length of glass tubing B about 45 cm. long. Fill the entire length, taking care to remove all air bubbles, by running water down the side of the tube from a pipette. Close with one finger, invert, and stand the lower end of the tube in a small vessel containing mercury. Clamp in position as shown in fig. 50.

As water is transpired from the leaves mercury will be drawn up the vertical tube. When it has reached a certain height air will be drawn into the tube through the air spaces of the stem and leaves, and further ascent stopped.

b. Suction Due to Evaporation. Take a porous pot (fig. 50 D), 7–8 cm. long, and join it to a vertical tube E by means of a rubber cork. Fill both with water, excluding all air, and clamp into position with the lower end of the tube dipping into mercury. As water is evaporated from the surface of the pot mercury will rise in the vertical tube.

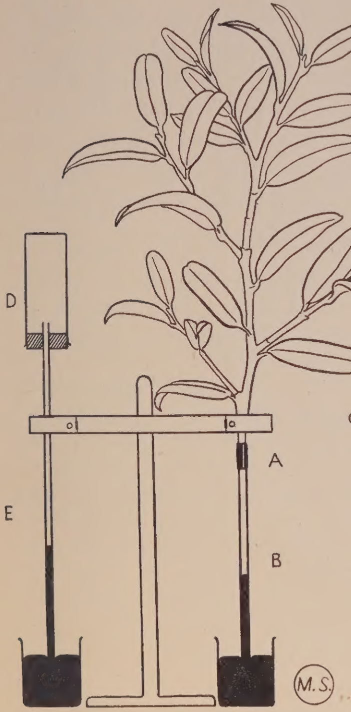


Fig. 50

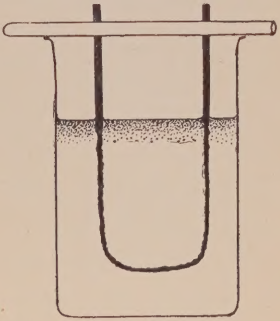


Fig. 51

Exp. 51. Osmotic Pressure

Coat the upper half of a parchment paper shell (*see* fig. 51) with paraffin wax to render it impermeable, and fit it with a wire or glass rod so that it can be suspended in a beaker.

Soak the shell and then pour a known quantity, say 25 c.c. of a molar solution of cane sugar into it. Suspend it in the beaker and add water to the latter until the liquids are at the same level inside and outside the membrane. All the unwaxed part of the membrane should be immersed. Allow to stand for 24 hours or longer, and then pour the solution in the shell into a narrow measuring cylinder. The volume will have increased to 35–40 c.c.

Exp. 52. PLASMOLYSIS

a. Cut a few sections from a fresh beet-root, and place in a watch-glass of water. Mount and examine under the microscope; the cells will appear full of sap. Immerse a section in a molar solution of sucrose, and leave for 5–10 minutes, then mount in the same medium and examine microscopically. Many of the cells will be plasmolysed, owing to the shrinking of their protoplasts as water passes out into the strong sugar solution.

b. Immerse similar sections in 5 per cent. glycerine. Plasmolysis will again take place, but after a time, 15 minutes or longer, the protoplasts will return to their normal position.

Glycerine slowly penetrates the protoplasts, and so increases the internal concentration of osmotically active substances. As this happens water re-enters the cell, and extends the protoplasm again.

To avoid the necessity for section-cutting filaments of *Spirogyra*, or other algae with conspicuous chloroplasts embedded in the cytoplasm, may be used.

Exp. 53. SUCTION PRESSURE. THE STRIP METHOD

Make a wall of plasticine, about 3 mm. deep, round the edges of two microscope slides. Lay the slides upon a sheet of squared (millimetre) paper. Fill one of the chambers with a 3 M calcium chloride solution, and the other with distilled water. Cut strips from the large petals of one of the species mentioned below, about 6 cm. long and 0·4–1·0 cm. wide. Measure the length and breadth accurately on the squared paper, and then immerse a strip in each of the liquids. Cover with another slide to prevent evaporation and measure the length and width again after an hour, and if possible after longer intervals.

The suction pressure of the strong solution will be greater than that of the petal, which will lose water and shrink (probably 3–5 mm.). In water owing to its greater suction pressure the petal will expand about 3 mm.

Ray florets of marguerite or other composites, and petals of *Begonia* or *Amaryllis* give good results.

Exp. 54. MEASUREMENT OF SUCTION PRESSURE

Make up a molecular solution of cane sugar by dissolving 85·5 gm. in 250 cc. of distilled water. From this prepare M/2, M/4, and M/8 solutions by successive dilutions, and put about 100 cc. of each into appropriately labelled wide-necked bottles. Cut a block from a potato with a cork borer, and cut it into slices 3 or 4 mm. thick. In this way prepare four lots of disks, each between 8 and 10 gm. in weight. Dry the cut surfaces with blotting-paper, but do not apply pressure. Weigh each lot and put one into each bottle. After 24–48 hours take out the disks, dry their surfaces as before, and weigh again. Some sets will show a gain in weight, owing to the uptake of water, and others a loss. Which set shows least change? In this the suction pressure of the solution is most nearly equal to that of the tissue.

The osmotic pressure of a molecular solution of sucrose = 26 atmospheres.

Exp. 55. THE PATH OF CONDUCTION

Uproot a young plant of balsam (*Impatiens*) in which the stem is less than a quarter of an inch thick in the lower internodes. Wash the soil away from the roots, and stand at once in a beaker containing about an inch of water-soluble eosin, or red ink. Allow to stand for one hour, then lift the plant out of the eosin, and wash the roots under a tap.

The xylem strands of the roots, stem and leaves will be picked out in bright red, and will be readily visible to the naked eye owing to the translucency of the tissues, and the position of the strands near the surface. Trace the course of the eosin from the roots to the minute strands of a leaf. If the experiment is carried on for a longer period the leaf mesophyll also becomes injected.

Cut a transverse section of an internode and mount under a microscope. It can then easily be seen that only the xylem vessels are stained, unless the experiment has been carried on a long time, when the dye will also have travelled into some of the adjacent cells. Wild balsams may be found as garden weeds in suitable condition during the summer months, and cultivated balsams can be raised without difficulty from seed from April onwards. Twigs of Portugal laurel (*Prunus lusitanica*) may also be used if sections are cut.

Exp. 56. PENETRATION BY METHYLENE BLUE

Fill a watch-glass with tap-water and stand it upon a piece of white paper. Add methylene blue drop by drop until the water is faintly tinted. Immerse a few threads of *Cladophora*, or other filamentous alga, in the water and cover with a second watch-glass to prevent drying up. Examine after 24 hours and notice that the water has become colourless again. If the alga is examined under a microscope it will be found to be deeply coloured by the dye, which has accumulated within the cells.

Thin slices of potato may also be used for this experiment.

VII

IRRITABILITY

The Nature of Irritability. The preceding chapters have described the various processes which lead to the synthesis of living protoplasm and so to the formation of the tissues and organs which together make a plant. The mature protoplast is not by any means an inert body, but is, on the contrary, characterized by its great physical and chemical activity, and one way in which this becomes apparent is through its ability to respond to changes of heat, light, pressure, moisture, and many other outside influences. The responses thus evoked are very varied according to their cause and the state of the protoplasm at the time of the change. Light alone has many different effects, being deadly to some protoplasts, such as those of *Bacillus tuberculosis*, and causing alterations in the rate of growth of nearly all plant organs. In addition, it effects further internal changes, such as the movements of chloroplasts, and prevents etiolation.

During photosynthesis light is taken up quantitatively, and is therefore called one of the *conditioning factors* of the process. In other cases, such as the bending of a stem towards a source of light, only a fraction of the energy used up is supplied by the light, which merely sets in train a long series of reactions in the protoplasts, these finally giving rise to the visible bending. When acting in this way, light is said to be a *stimulus*, and the protoplasm is said to *respond* in virtue of its *irritability*. Irritability is thus another name for the extreme reactiveness of protoplasm, which makes it always liable to change by influences from without, in addition to the many changes it undergoes spontaneously.

The energy imparted by the stimulus is often only a small

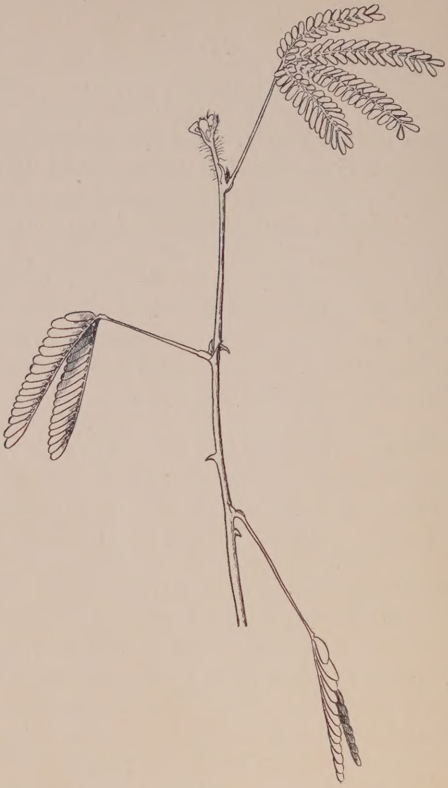


FIG. 52. A YOUNG PLANT OF *Mimosa pudica*

The top leaf is in the normal position, but the others have responded to a mechanical stimulus. The pulvini can be seen at the hinge positions. About natural size fraction of that used up in the response, the remainder being supplied by the protoplasm itself. For this reason, the plant's behaviour is sometimes likened to the action of a trigger, by which a small initial squeeze releases a very large amount of energy from the exploding cartridge. In the latter case, the amount of energy obtained from the explosion is not increased by increasing the pressure on the trigger, so there is no constant relation between the two; all that is required is that a certain minimum force should be applied, anything in excess of that amount having no further effect. Plant responses differ in this respect, since it has been found in those cases where measurements have been made that the amount of response is often proportional to the amount of stimulus. Trigger action cannot, therefore, be used as a name to describe the process.

The Internal Sequence. The visible response does not necessarily occur at the point where the stimulus first affects the plant, and hence it seems likely that some form of internal conduction occurs. There are thus three stages to be considered:

1. Induction ('Stimulation', 'Excitation') at the point acted upon by the stimulus.
2. Conduction, if any.
3. Response at the same point or some other.

Each of these stages is well illustrated by the rapid movements which occur in *Mimosa* leaves, when the leaf-tip is cut or slightly burnt. When the stimulus is applied to a leaf-tip, the leaflets close together in pairs, usually starting with those nearest to the affected tip, the closure being due to changes in shape of the swollen pulvini at the base of each leaflet. When the bottom pair has closed the response often occurs along other axes, and then starts from the bottom and works upwards, as the stimulating effect is conducted along them.

In the meantime, usually while the pinnae of the first axis are closing, the main leaf-stalk has dropped, the pulvinus at its base acting as hinge (fig. 52). These changes occur in a few seconds, but are not the full extent of the response, since the stalk and leaflets eventually return to their original position without any further external stimulus. The recovery movements take a much longer period than the original drooping, but both parts of the response use up energy, which is released from the protoplasm as a result of the stimulus.

The three stages are thus: (1) induction due to wounding at the leaf-tip, causing unknown changes in the affected cells, (2) rapid conduction along the leaf axes carrying stimuli to the various pulvini, and (3) response, consisting of a contraction of the lower sides of the pulvini, followed by a slow return to their original condition.

GEOTROPISM

Many of the known responses of plants have not yet been studied in detail, but a good example of one that has is given by the response of plant organs to gravity. This force exercises a mechanical drag on all parts of plants and produces visible effects in almost all of them, though these responses may sometimes be masked by the action of more powerful influences. The drag of gravity acts in a single direction only, and the response of the plant is a curvature which must bear some definite relation to the direction of the pull. Responses of this kind are called *tropisms*, and any tropism induced by gravity is named *geotropism*. The direction of the responsive turning is different in different organs; it may be towards the centre of the earth (*positive geotropism*), away from it (*negative geotropism*), or at right angles to this direction (*dia-geotropism*). The following list shows the usual responses of the various organs of plants:

1. Positively geotropic	primary roots; also some flower stalks and hypocotyls.
2. Negatively geotropic	primary stems; 'breathing' roots; fungal sporophores; coleoptiles and most hypocotyls.
3. Dia-geotropic	leaves; lateral roots and stems.
4. No response	tertiary roots; fungal hyphae.

It must be remembered, however, that the normal response may change during the life history of the organ, a striking example being afforded by the hypocotyl of *Adoxa Moschatellina*, which exhibits all three responses in turn. Upon germination the hypocotyl comes above ground owing to its negative geotropism; but shortly after it takes on a positive reaction, bending right over and again penetrating the ground. When it has become embedded in the soil it changes once more, becoming dia-geotropic, and so forms a rhizome which grows parallel with the surface of the ground.

Geotropic Response in Primary Roots. The behaviour of primary roots under gravitational stimulus was one of the first plant responses to be studied and its simpler features can easily be examined experimentally (**exp. 57**). If a young root is placed at an angle to the vertical its tip curves, as further growth takes place, sufficiently to bring it back into the plumb line. The older part of the root undergoes no visible change, curvature being restricted to the elongating region just behind the apex (fig. 53), and resulting from the greater increase of the cells on the upper side. The cells on the lower side also increase their length, but not to so great an extent as those on the upper.

If a root is placed for a short time at an angle to the vertical, and is then rotated and allowed to lie for an equal time at some other angle on the opposite side of the vertical, it will receive two unequal stimuli, and will eventually curve in the direction of the greater, if it is returned to a rest position. The amount of stimulus is proportional to the sine of the angle by which the root is deflected from the vertical, and it has been shown that the amount of induction or stimulation, but not necessarily the amount of response, is proportional to the same quantity. Thus geotropic stimulation is greatest when the root is placed horizontally (sine = 1) and nil when it is placed vertically (sine = 0). It is immaterial whether the root points directly downwards, or directly upwards, but in the latter case the slightest deviation will start a curvature which will eventually bring the root to the normal position of 'rest'.

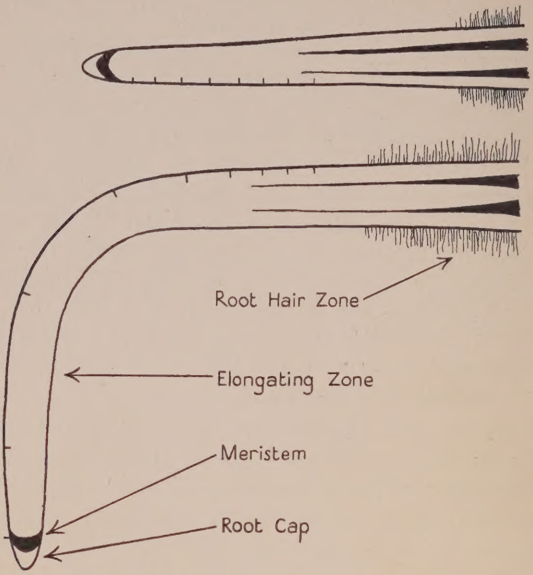


FIG. 53. GEOTROPIC CURVATURE OF A BEAN ROOT showing that response occurs in the elongating region. Diagrammatic. (M.S.)

The root does not sag inertly under its own weight, and this becomes apparent if it is first stimulated and then placed in a vertical direction before any response is visible. It does not then continue to grow in the vertical direction, but first forms a bend in the direction of the stimulation and only returns to the vertical in response to the second stimulation which results. The bending will also occur against a resistance, such as that of a viscous liquid, and work is, therefore, done, the necessary energy being released during respiration. It is, perhaps, due to this fact that there is no response in the absence of oxygen **(exp. 58)**.

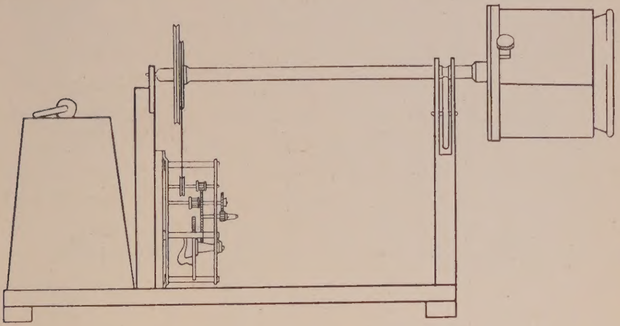


FIG. 54. A SIMPLE TYPE OF CLINOSTAT

The pot with the plant growing in it is clamped into the cubical box, which is made to rotate slowly by the clockwork. Two different speeds of rotation can be obtained by means of the two gear wheels on the spindle. (After Darwin)

The Clinostat. A root may be equally stimulated on both sides by placing it at two equal angles for equal times and in this case no curvature will result. The simplest way of securing a balance of stimuli is to place the plant upon a disk which is rotating slowly in the vertical plane; it then rests for an equal period in every angle to the vertical and in the course of a single rotation each side of the root is stimulated equally. A machine which secures this result is called a *clinostat* (fig. 54) and is of great service in studying geotropic responses. With an intermittent clinostat, i.e. one which can be stopped in any desired position for a known time, any amount of stimulus can be applied and its effects studied during a subsequent period of rotation.

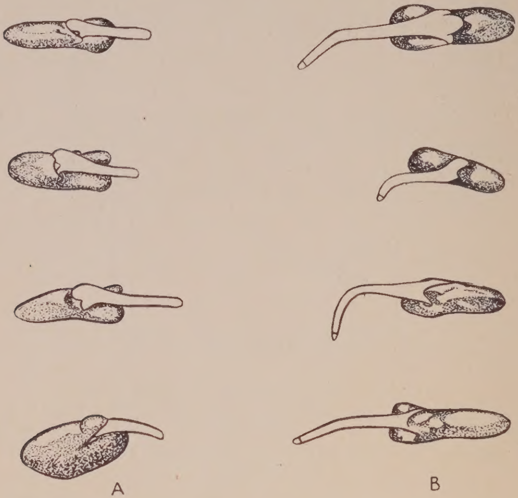


FIG. 55. GEOTROPIC RESPONSE OF DECAPITATED BEAN ROOTS
A. without tips; *B.* with tips replaced (drawn from a photograph by Snow)

Geotropic Induction in Roots. Although all parts of a root are subject to the geotropic pull of gravity, a limited region is specially 'sensitive' to its action. If the first two millimetres from the tip of a broad bean root is cut off, care being taken that the cut is accurately transverse, only a very slight curvature takes place when the root is placed in a horizontal position, even although the region which normally responds, i.e. the elongating zone, is still intact (*see* fig. 55 A). After a comparatively lengthy period, usually more than two days, the tip of the stump itself becomes sensitive owing to internal changes in its cells.

The nature of the changes which go on in the cells of the root-tip is not yet known, and it is probable that they are very complex, and perhaps very numerous. This chain of reactions which constitutes the induction phase is perhaps initiated by the rearrangement of comparatively heavy bodies, such as starch grains, which fall towards the lowest walls of the cells. A movement such as this, occurring under the influence of gravity, might well set in motion a series of reactions in so delicately adjusted a substance as protoplasm.

Conduction. It has just been explained that geotropic induction is greatest in a very short region at the apex, and that response occurs in the elongating region which extends for some distance behind this. It would seem, therefore, that the stimulus must be transmitted for a certain distance along the root or stem, and attempts have been made to discover how this is done. It is now generally believed that a definite chemical substance, formed or redistributed during the induction reactions at the tip, passes along the organ, until it reaches the cells in which changes are started, that finally produce the visible response. Substances which travel in the plant, and initiate important changes at a distance from the cells in which they were first formed are called *hormones*, and it is probable that they are very numerous and important in plants of complex organization as well as in animals. Animals are equipped with a special nervous tissue in addition, which conveys stimuli from one part to another, but microscopic examination has failed to reveal anything similar in plants, and the following experiments make its existence still more unlikely.

The sensitive tips of bean and other roots have been removed by an accurate transverse cut and then lightly replaced with a thin film of water or gelatine separating them from the stump. After being placed horizontally for about 24 hours, a large proportion curved down in a manner similar to that of normal roots (fig. 55). It is interesting to notice that the stumps of maize roots, similarly treated, carried out their usual curvature whether the tips placed upon them were those of roots or coleoptiles.

A similar experiment involving a different stimulus, light, has been performed with the coleoptiles of oats. The sensitive tips were again cut through and allowed to float freely upon the stumps. When a lateral beam of light was directed upon them curvature took place in the usual manner (p. 212). Yet another stimulus, mechanical shock to the leaf of *Mimosa*, can also be transmitted across a layer of water, as has been shown by severing a petiole and pushing the cut ends into a length of tubing, so that they were separated by a thin liquid layer. When the leaf-tip was burnt the usual drooping occurred at the base of the petiole on the farther side of the gap.

With these arrangements there could be no nervous connexions between the regions of induction and response, but it is quite possible that substances from the tips diffused through the water or gelatine and so into the stumps of the responding organs.

Geotropic Response in Primary Stems. The curvatures produced in primary stems are in the opposite direction to those produced in roots, that is to say, away from the earth's centre. The region of bending is, usually, similar to that in roots, being limited to the young elongating region behind the apex. The actual movement is rather more complicated, however, and results in the stem apex being carried beyond the upright position (fig. 56) to which it finally returns. The whole movement, to and fro, is the

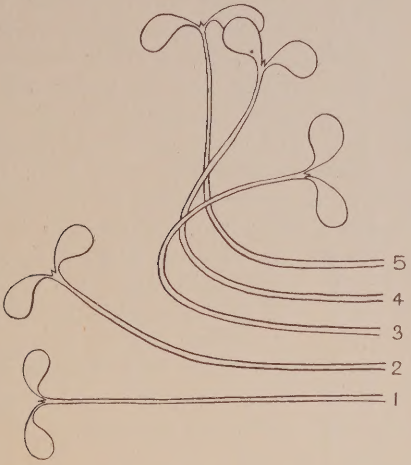


FIG. 56. DIAGRAM OF THE GEOTROPIC CURVATURE OF A YOUNG SHOOT

1. stem laid horizontal; 2–5 stages of response. (M.S.)

result of the single original stimulus, since it is carried out even if the plant is subsequently kept upon a clinostat. Geotropic response in stems is not entirely limited to the elongating zone of young and tender organs. Some woody twigs, particularly those of the Lombardy Poplar (*Populus nigra* var. *pyramidalis*), are able to carry out curvatures, and a more important instance is given by the nodes of grasses, cereals, carnations (*Dianthus caryophyllus*), etc. These retain their power of response long after the internodes on either side of them have lost it, and the result is that the stems of such plants which have been beaten down by the wind or rain return to the vertical position from a point near their base instead of only at the tip. Examples of this bending can frequently be seen in a field of young corn after bad weather, and a closer examination will show that the under surface of the node responsible for the recovery has become much longer than the upper, which may even have been compressed by the sharpness of the bend (fig. 57). If bending is prevented by slipping a glass tube round a horizontal stem, the rapid growth of the lower side causes it to form a swelling (fig. 65, and **exp. 60**). If the plant were rotated on a clinostat, the swelling would make a frill all round the node.

LIGHT AND PLANTS

Light has so many different effects upon plants, that it may be well to summarize them before describing the plant's responses in any detail; the following list gives each, including its technical name.

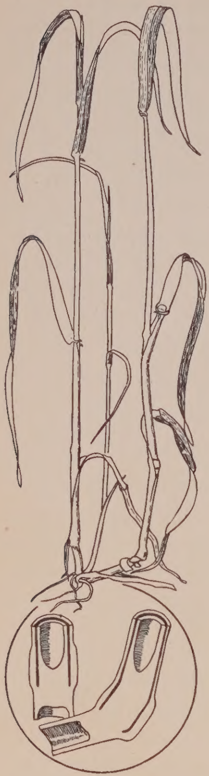


FIG. 57. YOUNG WHEAT PLANT

The stalk on the right has been knocked down and has returned to the upright position by the bending of its lower nodes. Other stalks in normal condition. Inset in circle is an enlarged view of nodes split down the centre. That on the left has remained vertical, that on the right shows curvature from the horizontal to the vertical position. Note the thickening of the lower side of the leaf sheath and the outgrowth of the stem

1. Photochemical reaction of photosynthesis.	Light absorbed to form sugars, etc. Includes action of light upon guard cells of stomata.
2. Chlorophyll formation.	
3. Effects upon form.	Prevention of etiolation. Production of rosette form.
4. Effects upon development.	Germination; time of flowering, etc.
5. Photonasty.	Responses to diffuse light, or with no definite relation to direction of light, e.g. 'sleep' movements of leaves and flowers.
6. Phototaxis.	Movement of chloroplasts and of free swimming cells relative to a source of light.
7. Phototropism.	Curvatures with definite relation to the direction of unilateral light.

The last three of these are usually classified as phenomena of irritability, and will be described in this chapter, though exact knowledge concerning phototaxis is rather slender and only a few of the more important points need be considered; photonasty and phototropism have been studied much more extensively.

PHOTONASTY

Periodic Movements of Flowers and Leaves. Many flowers open by day and close again at night, and a number of leaves exhibit similar motions in the so-called 'sleep' movements. Two causes are responsible in nearly all cases, light and heat, both of which are present to a greater degree during the day time than at night. It is often difficult to decide which of them is the stimulus causing any particular movement, but light is probably mainly responsible for the opening of daisy (*Bellis perennis*) heads, and wood sorrel (*Oxalis acetosella*) leaves; it also causes the closing of flowers such as Evening Primrose (*Oenothera*) and *Nicotiana*, and the drooping of balsam (*Impatiens*) leaves. The flower movements are most probably due to unequal growth on the two sides of the petals, while those of the leaves depend upon changes of turgor in the swollen pulvini at the hinge positions (cf. fig. 58).

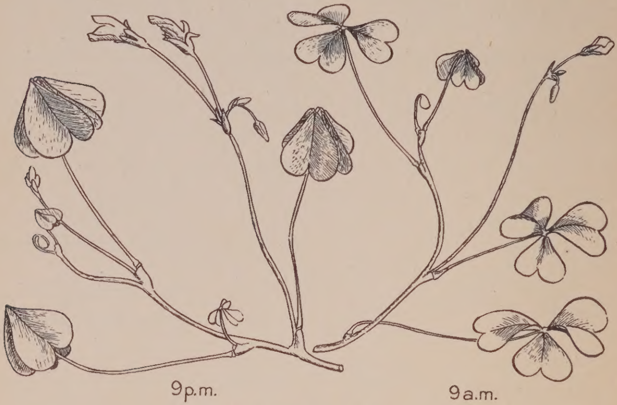


FIG. 58. *Oxalis corniculata*

Left a shoot drawn at 9 p.m. with the leaflets in the 'sleep' position owing to the loss of turgor by the pulvini at their point of attachment to the petioles. Right, a shoot drawn at 9 a.m. with the leaflets elevated at the 'light' position. Somewhat reduced

Changes in the Rate of Elongation of Roots and other Organs. The rate of elongation of roots, stems, and other organs such as hypocotyls, coleoptiles (p. 211), and fungal sporangiophores is often dependent upon the presence or absence of light, though some are unaffected. It is well known, for example, that shoots growing in the dark run to a much greater length than when grown normally in the light, even though they have no opportunity of synthesizing new material and so increasing the total amount of matter which they contain. The relation between light and the rate at which comparatively simple organs, such as young roots, increase their length has been carefully observed. Red light is without effect, and can be used to make measurements when white light is excluded, so that differences caused by the latter can be determined.

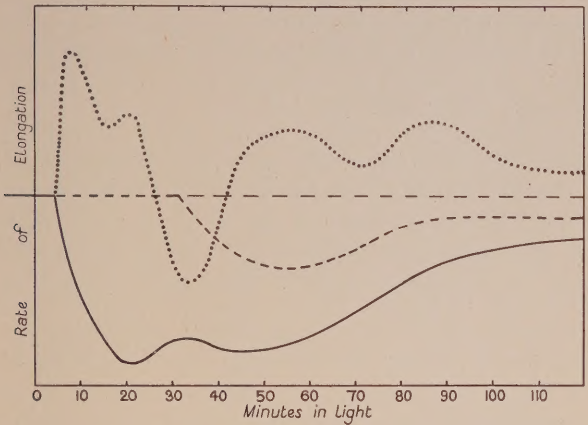


Fig. 59. Graph of the effect of light upon the elongation rates of *Sinapis* root (broken line); *Helianthus* hypocotyl (continuous line); *Phycomyces* sporangiophore (dotted line). Figures along the horizontal axis show the number of minutes in white light. (After Blaauw, modified)

A root of *Sinapis* kept under red light increased its length at a steady rate, which was carefully determined (fig. 59). When a weak white light was turned on, there was no visible change for half an hour, but at the end of that time elongation became perceptibly slower, though it became rather more rapid again after a further interval. Finally it settled down to a steady rate, slightly below that which occurred when white light was absent. The initial period during which no response was visible was presumably occupied by internal induction changes, and for this reason is given the name of *induction period*, and similar intervals of greater or lesser length occur in all responses to stimulation, whatever the nature of the stimulus.

The hypocotyl of a sunflower seedling (*Helianthus annuus*) was found to behave in a very similar way, the principal difference being that the induction period was much shorter, about three minutes instead of thirty. The coleoptile of the oat (*Avena sativa*) and the sporangiophore of *Phycomyces* were also found to respond in a complicated fashion. The oscillations of the elongating rate in the latter case were even more numerous, and the final steady rate in light was actually faster instead of slower than the 'dark' rate, unless the intensity was very high. Similar results were also obtained if only a flash of light was given instead of continuous illumination, and it could then be shown that the amount of response, or change in elongation rate, was proportional to the amount of light received (=intensity × duration of the flash).

PHOTOTAXIS

Free Swimming Cells. Many unicellular plants such as the *Volvocaceae* and swarm spores such as those of filamentous green algae are able to move about owing to the lashing of their cilia, the direction of their motion frequently depending upon the direction of the light falling upon them. Usually they move towards the source, providing it is not too bright (**exp. 63**) and away from it if it exceeds a certain high intensity, but a few species move away from lights of even medium intensity.

The slime fungi (*Myxomycetes*), which consist of naked protoplasm, and exhibit a gradual flowing motion, also move away from light sources.

Chloroplasts. In the leaves of many plants the chloroplasts are able to take up different positions in the assimilating cells according to the brightness of the illumination. In a simple organ, such as the assimilating tissue of duckweed (*Lemna trisulca*), which floats upon the surface of ponds, these arrangements are easily seen. In a moderate light the disk-shaped chloroplasts take up their position against the horizontal walls, so exposing a maximum amount of surface to the light falling from above, but under very strong illumination the chloroplasts migrate to the vertical walls and leave only the edges of their thickened disks pointing upwards. In darkness the chloroplasts are scattered between the two positions, and this applies also to the chloroplasts in the palisade tissue of a complex leaf, in which there is much reflection from the cell walls. If the light is made to fall exactly at right angles to the leaf surface, and so pass straight down the length of the palisade cells, the chloroplasts tend to desert the long side walls and collect at the upper and lower ends. A very intense light has the opposite effect, and the movement is then towards

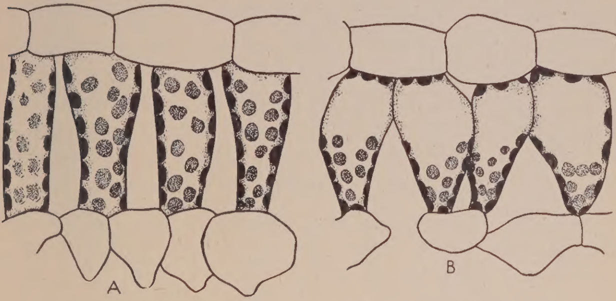


FIG. 60. PALISADE CELLS OF DWARF BEAN (*Phaseolus vulgaris*) showing the position of the chloroplasts in diffuse daylight (*A*), and in parallel light falling at right angles to the leaf surface (*B*). × 300–400. (After Senn, modified)

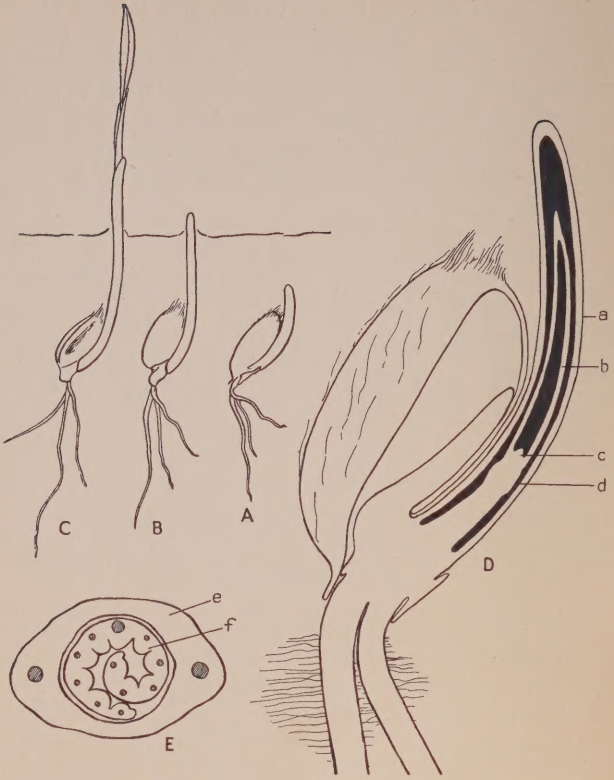


FIG. 61. COLEOPTILE OF WHEAT

A, *B*, *C*, stages in the germination of a wheat grain, showing the emergence of the coleoptile (*A*), coleoptile breaking through the soil surface (*B*), and the first leaf emerging from the coleoptile (*C*). *D*. enlarged section of *A*. *a*. coleoptile; *b*. first leaf; *c*. stem apex; *d*. node. *E*. transverse section of the sprout. *e*. coleoptile; *f*. the first leaf, vascular tissue shaded. (*E*. after Percival) the vertical walls, leaving the end ones bare. The mechanism of these movements is at present unknown, but they may be due to a streaming of the protoplasm carrying the chloroplasts with it.

PHOTOTROPISM

Just as in the case of geotropism, the curvatures which occur in response to a lateral light differ in different plants and organs, and may be classified according to their direction relative to the path of light. An organ which bends towards the source is said to be positively phototropic, while one which bends away from it is called negatively phototropic, and if a position is taken up at right angles to the path of light the organ is said to be transversely or dia-phototropic. The commonest examples are as follows:

1. Positively phototropic.	Primary stems; coleoptiles of grasses, etc.; sporangiophores of many fungi.
2. Negatively phototropic.	Some primary roots.
3. Dia-phototropic.	Many leaves.
4. No response.	Some roots.

Responses in Roots and Stems. These are easily observed by growing seedlings under lateral illumination (**exp. 64**); like the responses of geotropism, they are limited to the young elongating zones, and result from the more rapid extension of the side away from the source of light. Once a curvature has been made, it is never straightened out; if the direction of the stimulus is altered, the second response occurs beyond the first owing to the growth of the organ meanwhile and the consequent shifting of the responding region.

Coleoptiles. The coleoptile of the Gramineae is particularly reactive to light, and has proved especially useful in the study of phototropism. It probably represents the first leaf of the seedling, but it has the special form of a hollow cylinder, inside which the first normal leaf develops (fig. 61). It is solid at the tip and grows to a fixed length by the elongation of the cells lying 3–5 cm. behind. In consequence, it curves comparatively low down, not at the top, and in this respect differs from the stem which is developed later.

The amount of response to a given stimulus can be measured by the angle to which the coleoptile bends or by the time taken to reach a fixed angle from the original vertical position. It is also possible to control the amount of light supplied very accurately, and when this is done it is found that the amount of response is strictly proportional to the amount of light reaching the plant. The intensity of the light is not important, unless it is made so high that a reversed response is caused, as in phototaxis. In all usual cases, a strong light applied for a short time has the same effect as a weak light given for a sufficiently long time.

Two stimuli can be balanced against one another by supplying equal quantities in exactly opposite directions, and if this is done no curvature results. It is interesting to notice that the stimuli need not be of the same kind, and a light stimulus could, for example, be balanced by a gravitational one. Under natural conditions the light effect is usually the greater, so that a negatively geotropic stem curves towards the light, if this happens to fall from one side only.

Phototropic Induction. If the top 2 mm. of a coleoptile are cut off, it loses its reactiveness to light, even though the bending region lower down is unharmed, but if the upper part is merely covered with a cap, response is much reduced but not destroyed entirely (fig. 62). This difference is explained by the supposition that the substance which causes elongation is formed in the tip alone, but that light may cause its redistribution either in the tip or in the lower parts into which it penetrates. Thus when the tip is removed there is no response because nothing can pass out of it into the stump, but when the tip is merely covered the essential material can still pass, becoming redistributed during the later stages of its movement. The nature of the induction changes can only be guessed at, though it is reasonable to suppose that they begin with a photochemical reaction.

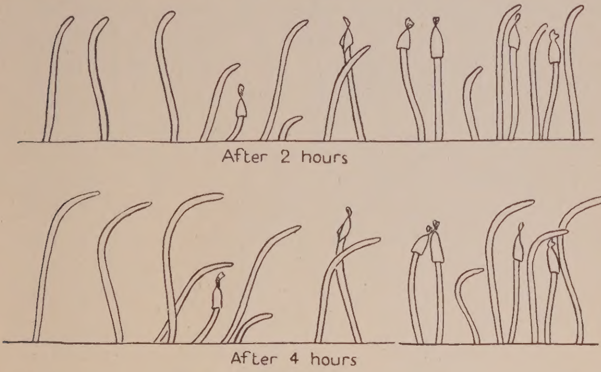


FIG. 62. COLEOPTILES OF OAT

grown in the dark and then subjected to a lateral beam of light. Marked curvature is shown by those with uncovered tips. Those with tin foil caps show much slighter curvatures

Phototropic Responses of Leaves. Most leaves in their younger stages take up definite positions in relation to the direction of the light which they receive. These adjustments generally depend upon unequal rates of growth in the petiole, which by forming twists bring the leaf laminae into their final position. The power of elongating becomes much less or is entirely lost as the petiole grows older, and the ability to carry out these light responses goes with it.

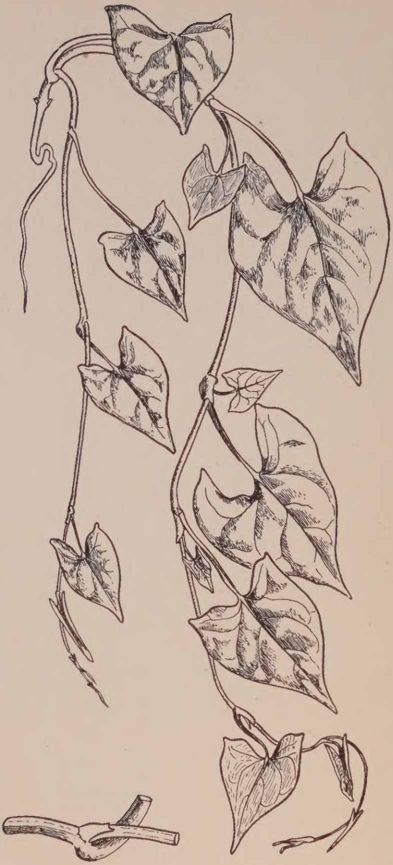


FIG. 63. SEEDLING PLANT OF POLYGONUM CONVOLVULUS trailing over the surface of the ground. All the leaves have come into the horizontal position, regardless of their insertion on the stem, owing to the twisting of their petioles. About natural size. Insertion of one petiole shown enlarged

As a result of this the leaf becomes permanently fixed in the position it has taken up, and light changes in the older stages have no visible effect. If a creeping branch of *Convolvulus*, *Calystegia*, etc. (fig. 63), is examined, it will be seen that although the petioles arise from the stem in different ranks, they are twisted in such a way that all the laminae lie horizontally or approximately at right angles to the path of the light falling upon them.

Some plants have the opposite arrangement, their leaves being placed with their edges towards the direction of the strongest or midday light. The well-known *compass plants* (*Lactuca scariola*, etc.), which have their edges pointing due north and south, are examples of this kind (fig. 64).

In *Robinia pseudacacia* the leaf movements depend upon turgor changes and are more elaborate; if the light is very strong they take up a profile position exposing only their edges to the direct rays, but in more moderate intensities they take up the customary transverse position. In this species, as in many others with pulvini, the power of response is retained in leaves which are fully grown, and frequent adjustments go on.

The biological consequences of all these leaf arrangements are interesting. Their general effect is to secure for the leaf the maximum amount of light of moderate intensity and to avoid that of high intensity. The former is effective in carbon assimilation and the latter is apt to cause a breakdown in the photosynthetic machinery, as well as a wider opening of the stomata and higher internal temperatures, which may have an adverse effect in hot or dry climates. It must be remembered, however, that the leaves of many plants growing under such conditions do not exhibit any of these directional effects and manage quite well without them. It is, in fact, very easy to credit such 'adaptations' with more biological importance than they really possess.

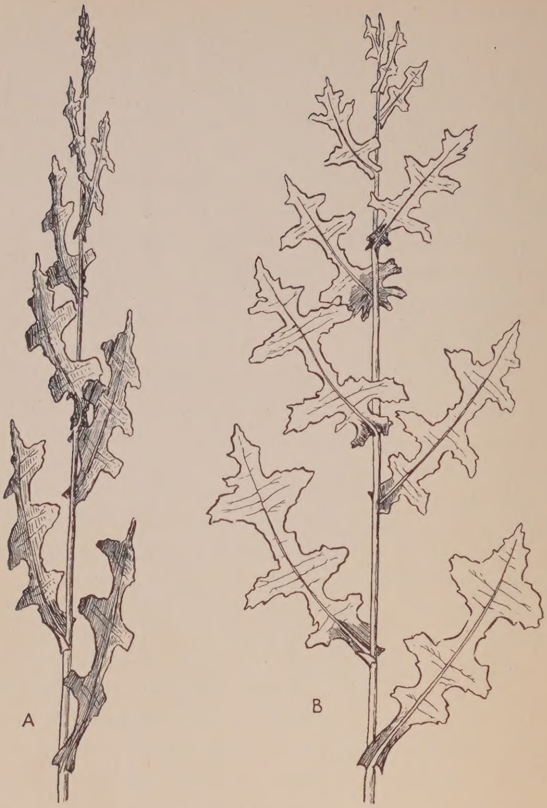


FIG. 64. *Lactuca scariola*
A. seen from south or north; *B*. seen from east or west.
(After Kerner)

EXPERIMENTAL WORK

Exp. 57. THE REGION OF GEOTROPIC RESPONSE

Germinate some broad beans in a mixture of equal parts of silver sand and fine sawdust, which should be kept moist but not sodden. When the roots are about ¾ in.–1 in. long, remove three of them, and mark equal divisions (about 2 mm.) along the root. This is conveniently done by drawing out a fine thread of brunswick black between two pins and then dropping it across the root. The mark thus made adheres well when dry, and causes no damage to the tissues. By means of long wires or pins fix the two beans to the cork of a wide necked bottle lined with damp blotting-paper; when finally placed in position they should have their roots and flat sides horizontal. Put some water in the bottom of the bottle, insert the cork and leave over night. By the following morning response will be practically complete.

Measure the lengths now shown by each of the original divisions and notice that bending has occurred in the region of the greatest elongation (cf. fig. 53).

Exp. 58. OXYGEN NECESSARY FOR RESPONSE

Take one or two beans germinated as in the previous experiment, and fix them below the cork of a wide necked bottle by means of long pins, so that their roots and flat sides are horizontal. Clamp in this position outside the bottle for thirty minutes. Partly fill the bottle with an alkaline solution of pyrogallol, and then put in the beans, still fixed to the cork, which must fit tightly and should be painted with a thin coating of wax. The beans should be as near the liquid as possible without touching it.

The pyrogallol will rapidly absorb the oxygen above its surface, and it will be noticed that the bean roots show no curvature after a period which sufficed for marked response in **exp. 57.**

Exp. 59. Reaction Time

Rule a horizontal straight line on a piece of cardboard, and fix the latter in a vertical position. Then fasten a young sunflower seedling to the board so that the root lies along the line. Keep under close observation, and note the time taken for the first appearance of response. This is usually a few minutes.

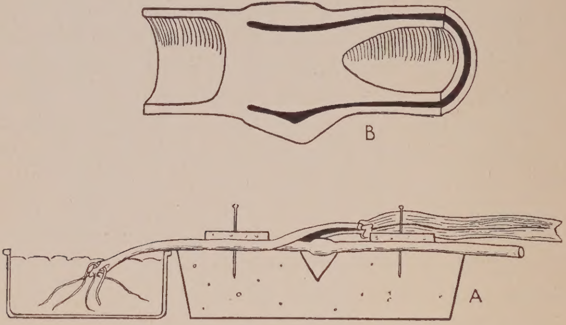


Fig. 65

Exp. 60. Geotropic Responses of Grass Nodes

Collect some long grass or cereal stalks with roots attached, selecting those which have prominent nodes. Prepare a large flat cork by cutting a shallow groove across it, and fasten a stalk horizontally with the node above the groove by means of a piece of sheet cork or cardboard on either side (fig. 65 a). Fix a second stalk only on the side nearest the roots, leaving the upper end free to move. Embed the roots of both in damp sawdust and keep thoroughly moist for a week.

The second stalk being free to move above the node will have returned to the vertical position owing to the greater growth of the node's lower surface. If the outer leaf sheaths are stripped away it will be found that the unequal growth of the fixed node has formed a swelling on its under side (fig. 65 B).

Exp. 61. CURVATURE IN A REST POSITION AFTER STIMULATION

Take three broad beans germinated as in **exp. 57** and lay them on their side for 30 minutes. Mark their upper surfaces and then plant them again in the sand and sawdust, taking care that the roots are vertical. Examine again after 24 hours, when it will be found that the root has curved away from the marked side, i.e. in the direction of the stimulus.

Exp. 62. THE CLINOSTAT

For construction of the apparatus see fig. 54. If a clinostat is not available, a simple one, sufficient for light weights, may be improvized from a cheap clock by removing the hands and fixing a thin rod to the 'minute' spindle. (A watch-mender will do this.) The rod should be passed through a cork and its far end supported in a hole drilled in a piece of hard wood or metal, the latter being supported by a stand. The cork may advantageously be covered with blotting-paper which can be kept moist.

a. Stimulate some broad beans as in the previous experiment, but instead of planting them in sawdust fix them to the cork with their roots parallel to the revolving rod. It is important to see that they are kept moist. After about 24 hours the roots will again have curved in the direction of the original stimulus, and the small stimuli received while revolving slowly on the clinostat will cancel one another out. This method of examining the effect of the initial stimulus is more reliable than that of exp. 61, since in that case any slight deviation from the vertical will lead to a further curvature, which may mask the primary response.

b. Fix a grass stalk with well-developed nodes on to a clinostat parallel to the axis of rotation. Keep it there for a week, during which period the roots must be kept moist with damp cotton-wool pinned to the clinostat. An automatic dripper can be arranged by corking a cotton-wool wick into the neck of a bottle of water and supporting it horizontally.

If the nodes are examined at the end of the experiment, they will be found to have developed a ring of tissue all round instead of on one side only as in **exp. 60.**

Exp. 63. PHOTOTAXIS

Introduce sufficient motile algae to give a green coloration into a beaker, darkened with brown paper and having a slit about $\frac{1}{8}$ in. broad on one side. Allow to stand in a bright window. At the end of the day strip away the paper, and the region of the slit will then be found to be coloured green by the algae which have collected there, whereas the darker sides will be free from them.

Large numbers of *Chlamydomonas* cells can usually be found in water butts or other fresh standing water during the summer. As an alternative *Euglena* may be found in stagnant ponds in summer and autumn.

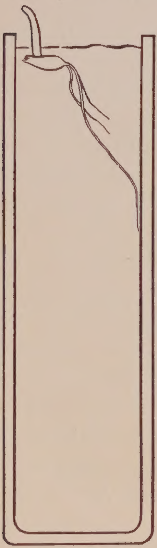


FIG. 66

Exp. 64. PHOTOTROPISM

Soak some oat grains, and plant in damp soil in a glass box (fig. 66). The soil must be crumbly and not too tightly packed and the grains should be near the surface and along one of the longer edges of the box. Place in a bright window and keep moist for a week to ten days. The coleoptiles as they emerge will curve towards the light, and the rootlets will become visible on the darker side of the box away from the window. If a similar experiment is performed with mustard seed, the phototropic curvature of the young stems will also be observed.

Exp. 65. THE TIP, THE SENSITIVE REGION OF COLEOPTILES

Soak about two dozen oat grains and put them to germinate in a pot of damp soil. The pot must be kept in the dark until the coleoptiles are half to three-quarters of an inch high (3–4 days). At this stage cut the top two millimetres from about half the plants. This operation must be carried out in red light. Place a cap of dull black paper with a slit in one side over the pot without damaging the seedlings and illuminate by means of an ordinary electric lamp placed about 1 foot from the slit. Turn out this light and examine the seedlings by red light, or very dull daylight, after 2 hours and again after 4 hours illumination. The uncut coleoptiles will have curved towards the light, whereas the others will remain upright.

VIII

GROWTH

THE GROWTH OF UNICELLULAR PLANTS

ALL plants, the simplest as well as the most intricate, are slowly changing the whole time they are alive. They do not come into existence in a mature form remaining unchanged for the whole of their lives, but show a gradual and regular development leading up to maturity and reproduction. In free living single cells the life-history may be very simple, starting with a small cell formed by the division of a large mother cell, the daughter increasing its size until it has reached that of its parent, and then dividing in its turn. Even in unicellular plants, however, this simple cycle may be varied; pairs of cells may conjugate, or the organism may form resistant walls, and go into a state of inertia, suspending the normal development, perhaps for long periods. The different phases of development are determined very largely by the inherent nature of the plant, but they are also affected by external factors. Thus lack of moisture leads to the formation of resting spores, and the intensity of light often has a strong influence on the frequency of reproduction. The cells in a *Spirogyra* filament were found to divide more rapidly when they were subjected to a stronger light, though their individual growth was considerably reduced; with *Protosiphon* the result was just the reverse.

The Growth of a Colony. If a few yeast cells are put into an aerated sugar solution, containing in addition the other elements necessary for growth, development sets in at once. As soon as each cell has reached a certain size (about 10 μ in diameter) it buds off a daughter cell (fig. 67) and so increases the total number of individuals present in the solution. Under favourable conditions this would happen about once in every hour, and this period would then be

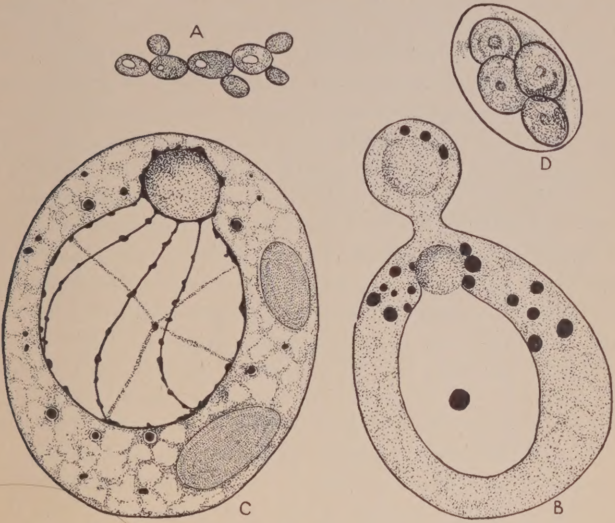


FIG. 67. YEAST

A. cells budding and forming chains, × 200
B. single cell forming bud
C. diagram of the structure of an individual cell
D. formation of spores inside mother cell, this takes place under unfavourable conditions and a resting stage follows. (*B* and *C* after Wager and Penistone; *D*. after Curtis)

called the *generation time*. The other cells present will also behave in a similar fashion, but the divisions will not all be simultaneous, because when first put into the solution the cells were not all at the same stage of development, and there will also be small individual differences between them. On the average, however, the total number of cells in the solution would be doubled at the end of each succeeding generation time. Assuming this to be an hour, each original cell in the culture by the end of the day would have given rise to 16·5 millions, and at the end of a second day to 281·5 millions. This unrestricted development could only go on as long as there was sufficient sugar and other materials available, and in a limited environment, such as a culture flask, a deficiency would soon set in. The rate of growth of the colony would then slow down as more and more of the cells were forced into an inert condition, and a correspondingly smaller number continued to divide. In actual practice the growth of a yeast colony is retarded even before a deficiency of nutrients becomes felt, and this is due to the accumulation of alcohol, which the yeast itself produces. In a concentration of about 15 per cent. this brings all activity to a standstill, and it may slow down growth considerably at much lower values.

There are thus two phases in the growth of a colony of free cells, (1) the phase of *unrestricted growth*, in which all the cells are dividing at more or less equal intervals, and (2) the *senescent* phase, in which for one reason or another increasing numbers of the cells become inactive. If the cells are inactive when first introduced into the culture, there is also an *initial lag phase* during which they come to their full activity.

HIGHER PLANTS

Correlation and Inhibition. All complex plants consist of large numbers of individual cells, but they differ from simple colonies, because the cells are no longer independent of one another, and are even linked by the protoplasmic fibrils, which pass through their walls (*see* fig. 29). The effects which individual cells within higher plants exercise upon other cells of the same plant are very complex and only slightly explored at present; the following are common examples shown by the bean plant.

If the root is cut away from the stem, cells near the cut surface start to divide, and eventually form new roots. In other words, the removal of the original root has taken away some influence, or *inhibition*, which prevented these cells from forming other roots while it was present.

A bean stem usually develops by means of an apical bud, and so grows into a single tall stem, though lateral buds are also formed in the axils of the leaves, remaining dormant as long as the tip of the stalk is intact. If the young leaves near the tip are removed, even though the growing point is not damaged, the dormant axillary buds begin to develop, and form side shoots, whose further growth may be stopped again as the terminal bud produces new leaves in place of those cut away. It seems, therefore, that these leaves exercise some effect upon the cells of the side buds, which prevents their development. It is thought that inhibitions of this kind are caused by the formation of some substance in the young leaves, which travels down the stem, or alternatively, begins a series of changes extending down it, and that these on reaching the axillary buds prevent their growth.

The Life-Cycle. Since all plants start from simple beginnings, the life-cycle becomes more complex with increasing complexity of structure, and stages of development are introduced, which have no analogy in the simpler examples. Flowering plants, the most complex as well as the most important group of the vegetable kingdom, have a cycle which is quite characteristic, although it undergoes many modifications in different members of the group. The adult plant forms seeds, consisting essentially of an embryo and tissues containing abundant reserves. The majority of seeds become dry when they are ripe, and in this state can remain with hardly any change often for many years. Sooner or later they take up moisture and germinate, the young plant depending at first upon the accumulated materials in its seed, but afterwards attaining the power of assimilating nutrient materials from the environment. There follows a period of vegetative growth during which leaves, stems, and roots are increased in number and size, and finally a reproductive phase is reached in which the new plant forms seed in its turn.

Annuals and Perennials. In annual plants these changes all occur in the course of a single season, and may take no more than a few weeks to come to completion; the ephemeral weeds which spring up on cultivated land illustrate such a life-cycle.

Perennial plants, on the other hand, persist as individuals from one year to another, and may even attain extreme ages, such as those of yew trees (*Taxus baccata*), which it is said may exceed a thousand years, and the big trees of California, which exceed three thousand. The life story of perennials is more complex than that of annuals, because instead of having a single season of vegetative growth closed by a period of reproduction, they have a succession of such phases separated by periods of comparative inactivity or *dormancy*. The resumption of active growth after dormancy has many physiological features in common with germination, but also important differences associated with the fact that dormant plants are not usually as dry as resting seeds.

THE FORMATION OF SEEDS

Rapid and visible growth begins in higher plants with the germination of the seed, but, when this occurs, the young plant or embryo has already been in existence some time, since it originates with the fertilization of an egg within an ovule of the parent flower. This single cell divides several times to form the embryo in which cotyledons and rudimentary axes, made up principally by their growing points, are distinguishable. Simultaneously with these early cell divisions in the embryo other tissues are formed which enclose the embryo, and in some of these, or in the cotyledons themselves, large quantities of reserve substances accumulate. These pass in from the parent plant in water soluble forms, and are precipitated in the seed tissues throughout the period of maturation. Thus amino-acids give rise to insoluble proteins, which occur in all seeds, some of them entering into complex combination with phosphates to form the conjugated, or nucleo-proteins. At first, however, nucleo-proteins are comparatively scarce, most of the phosphates being precipitated in various other organic forms, all of them very insoluble. Other mineral substances behave similarly, and as long as the seeds are developing there is a continuous movement of the simpler essential materials in their direction. Among these substances the sugars are important, as they provide the source of energy which is subsequently drawn upon during germination. They do not accumulate unchanged, however, but in the great majority of seeds are transformed into fats; in others, particularly among the cereals (*Gramineae*) and legumes (*Leguminosae*), they are precipitated as starch.

Dormancy of Seeds. When the building up of the seed is complete, germination in some species follows at once, but much more frequently there is a period of dormancy, even if the external conditions favour active development. During this period respiration and all other activities are reduced almost to a standstill. The state of the protoplasts becomes much more static than at any other time, and they tend to lose their normal characteristic of continuous change. It is impossible to say whether activity stops entirely, but it is known that it may be reduced to an extreme slowness without destroying the ability of the seeds to germinate afterwards. This has been done experimentally by drying them as thoroughly as possible, keeping them in a vacuum for years, or subjecting them to a temperature of −250° C., and after any of these treatments they have still been able to germinate.

Sensational claims have sometimes been made as to the length of time for which dormant seeds retain their ability to germinate. Actually this period varies very much in different species, and particularly among water plants may be very short, often only a few weeks. Grains of wheat have never been found to survive for more than sixteen years in spite of fraudulent stories about living seeds from the granaries of Herculaneum or Egyptian tombs. Seeds of the *Leguminosae* may germinate after considerably longer periods, and the extreme example is that of *Cassia bicapsularis*, ten seeds of which were tried after they had lain for eighty-seven years in an old herbarium. Three of these seeds germinated, but seeds of the same family kept in the moist condition by sinking them in bottles three feet deep in wet sand lost their ability to germinate within five years.

The great majority of dormant seeds are characterized by their dryness, having only about 10 to 20 per cent. of water instead of the 80 to 90 per cent. which is present in more active tissues. 'Wet dormancy', although less frequent, is not, however, unknown even in thin-walled seeds, where there is no doubt that the water penetrates to the interior. It is found especially among seeds falling into mud, or in forest soils, where abundant carbon dioxide is being liberated by the soil bacteria, the carbon dioxide acting as a narcotic.

Causes of Seed Dormancy. As in the last example, external conditions sometimes prevent seeds from germinating, and so keep them dormant until there is a change. Dormancy frequently arises, on the other hand, from internal causes, three of which may be named. *Incomplete development of the embryo* is frequent in such common species as Lesser Celandine (*Ranunculus ficaria*) and Wood Anemone (*Anemone nemorosa*). In such instances, although the seed is to all external appearances ripe when it is shed from the parent plant, the embryo itself is only partially differentiated, and undergoes further development while the seed is lying in the ground. *After-ripening* is characteristic of Juniper and other species in which the embryo is apparently complete at the time of seed dispersal, but still unable to germinate. During the further period of maturation which is necessary in such seeds, various internal changes are going on, especially an increasing activity of the enzymes. Finally, *impermeable seed-coats* may also be a cause of dormancy by preventing the access of water or even of air to the embryo.

GERMINATION

Dry seeds are very hygroscopic, and the first event in germination is the uptake of large quantities of water, which is imbibed into the protoplast or passes into the cell vacuole. In doing this the seeds, which at first are more or less wrinkled and shrunken in appearance, increase their size and become turgid, stretching out the folds in the seed coat. At the same time the total weight of the seed is increased by that of the water, which now forms a high proportion of the whole (**exp. 68**). The suction pressure of the seeds is very high until their colloids are saturated, and if the testa is tight round the internal tissues, or inelastic, the swelling of the colloids which takes place during the imbibition will cause it to burst. This actually happens in some seeds, but in the majority the testa is sufficiently loose or elastic to allow the colloids to complete their swelling without breaking it. Indehiscent fruits containing a single seed often have hard shells and are rarely ruptured by such swellings, since the dry seed only occupies a fraction of the space inside them.

As soon as the seed tissues have become saturated the embryo starts into renewed growth inside them, and although no new solids are entering the seed, its own materials undergo extensive transformations. As the embryonic cells divide and grow the space occupied by them becomes much greater, and this enlargement does not come to an early stop like the swelling of the original colloids, but may continue more or less indefinitely. The pressure developed by the growth of the young cells may be very considerable, though not so great as might be supposed at first sight. The growth of the embryo of a hazel-nut (*Corylus avellana*) was found to cause a distending pressure of 50 lb. per sq. in., but the dry shell of the fruit could resist 120 lb. to the sq. in. before breaking. The resistance of the wall dropped very considerably when it was thoroughly wetted, however, and could then withstand no more than 45 lb. to the sq. in., and was easily ruptured by the growth of the embryo.

The resistance to splitting offered by the seed coat or fruit wall varies enormously, from the thin membranes of peas to the woody shells of coconuts or of *Canna indica*, whose seeds have even upon occasion been used as shot. In coconuts rupture occurs at special weak points, the 'plugs', but there are other seeds like those of *Canna* in which germination is completely held up until the walls have rotted.

In almost all cases the first organ to emerge from the split in the testa is the *radicle*, or embryonic root, and since it is positively geotropic it turns over and grows downwards no matter at what angle it first appears. The young shoot soon follows the radicle, and taking the opposite direction makes its way above ground, where it develops chlorophyll and commences active assimilation.

The apex of an adult stem is not suited to penetrating soil like the apex of a root, and special organs are sometimes formed, which enable the young stem to grow to the surface. The coleoptile of the Gramineae, which is morphologically the first leaf, forms a hollow tube, which is furnished with a solid tip, and is about five centimetres long (*see* fig. 61). The first normal leaf grows inside this special organ and emerges near the tip, when it has approached the surface of the ground. If the seed is planted at a depth greater than five or six centimetres the leaf remains curled up when it leaves the coleoptile until it reaches the light. It can be made to uncurl underground by planting the seed close to the glass side of a well-illuminated box, and when this is done it can no longer penetrate the soil but becomes twisted and folded as it uncurls (fig. 68).

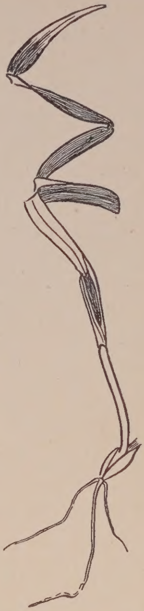


Fig. 68. Deeply planted wheat seedling germinating in soil against the side of an illuminated glass box. The light has caused the first leaf to appear from the coleoptile and unfold, with the result that it has become crumpled by the resistance of the soil

The shoots of many other plants, such as peas and beans, are curled over when they first leave the seed, so that only the back of the hook and not the actual apex is pressed against the soil. Here again, if the underground stem is illuminated, its emergence becomes uncertain due to the fact that the bend straightens out into the upright position, which it normally assumes only on leaving the soil.

The Biochemistry of Germination. The changes just described involve the expenditure of energy, and this is supplied by respiration, which is very rapid during germination and the early growth of the seedling. Sugars are consequently decomposed, and although the volume of the seedling increases, its weight is reduced by that of the carbon dioxide, which escapes into the atmosphere. Grains of wheat and maize may lose as much as half their total dry weight during this period, which for wheat is no more than six days. In both these seeds the reserve carbohydrates are in the form of starch, and fatty seeds generally lose a much smaller fraction of their weight, in sunflowers for example about one tenth, associated with the fact that fats release more energy per unit weight of material oxidized.

Whether the seeds have starchy or fatty reserves, the loss in weight is always due to loss of carbon, hydrogen, and oxygen; nitrogen is just as plentiful after germination as before, since it is not oxidized during respiration. The mineral elements are also unaltered in quantity, and ash from a sample of seedlings is just the same as ash from a sample of the ungerminated seed. This does not mean that these elements are inactive in the changes going on, and the compounds in which they exist undergo, in fact, numerous transformations. The biochemical changes which go on during germination may in very general outline be described as follows.

Starch and fats disappear from the storage tissues, becoming converted into sugars, some of which are broken down by respiration. Another fraction of the sugar formed in the storage tissues travels into the growing cells of the embryo, and is built up into new cell material, especially the cellulose of the newly-formed walls, or is again precipitated as starch.

Fig. 69 shows these changes in a potato seed and its seedling, the original reserve being in this case fat, which forms a quarter of the total weight of the seed. Starch is entirely absent in the dry state, although it is present in small quantities in most fatty seeds. As soon as water has been absorbed the oil droplets in the endosperm begin to disappear. And when the young root is thrust out through the testa, a regular arrangement of the principal reserve materials can be discovered. Nearest the seed, fat is still the main accumulation, but further along it is replaced by starch, and near the tip this may in turn be replaced by sugars.

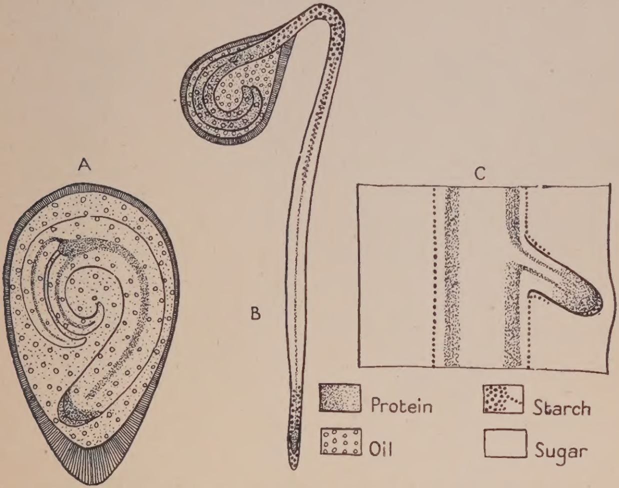


FIG. 69. Potato seed (*A*) and seedling (*B*) showing the distribution of the principal reserves; *C*. longitudinal section of a piece of root showing the origin of a lateral rootlet. (*A* and *B* redrawn after de Vries)

While these changes have been going on the insoluble proteins present in the endosperm have been converted into forms soluble in salt solutions and water. The various products of protein hydrolysis, peptones, polypeptides, and amino-acids, appear simultaneously as a result of the activity of the proteases, which, in common with other enzyme activities, increases markedly at this time. The amino-acids and perhaps other soluble forms are translocated towards the growing points of the embryo, and there give rise to the proteins of the new tissues, which consist very largely of the conjugated nucleo-proteins. The distribution of these various substances in a castor-oil (*Ricinus communis*) seed about to germinate is shown in fig. 70; in a dry seed peptones, polypeptides, and amino-acids are absent.

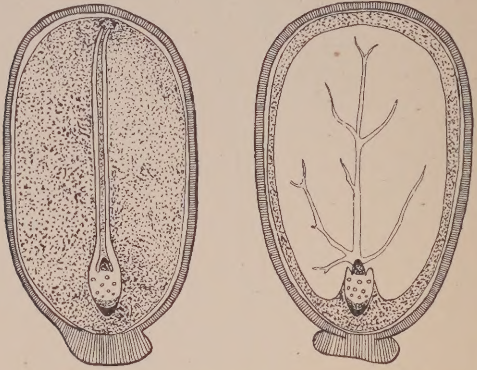


FIG. 70. SOAKED CASTOR OIL SEED READY TO GERMINATE
showing the distribution of proteins and related substances. Left in plane at right angles to the cotyledons; right parallel to cotyledons. Tissues with nucleo-proteins in full black; soluble proteins, dot shading; peptones and polypeptides, circles; amino-acids, white

When the radicle has emerged proteins are always abundant in the meristem, remaining plentiful in this position as long as the root lives; the meristems of lateral roots show similar accumulations, even while they are penetrating the parent cortex (fig. 69 c). In slightly older roots a regular succession of reserves can be made out in passing from the tip backwards. The root-cap cells are always full of starch, while the elongating region and the older parts of the root contain sugars. The arrangement of the nitrogenous substances within the protein region at the tip of the young radicle is probably similar to that shown in the embryo of *Ricinus*, that is to say, nucleo-proteins predominate at the tip immediately behind the root-cap, and are followed by proteins, peptones, and amino-acids in that order.

Most of the mineral elements present as compounds in the reserve tissues show a rather similar behaviour to that of nitrogen and carbon. Their insoluble compounds become converted, that is to say, into soluble forms which are translocated into the embryonic meristems, and there form fresh compounds in the growing tissues. The best-known example is phosphorus, which is present in the dry seed as lecithin and various insoluble organic phosphates. During germination these substances break down, and the comparatively soluble inorganic phosphates appear temporarily in their place, and pass by degrees into the embryo. In the meristems they give rise to nucleic acid, an organic phosphorus compound, which unites with simple proteins to form nucleo-proteins. Potassium differs from phosphorus and the majority of other elements by existing in the plant always in soluble forms, whatever the stage of development.

EXTERNAL CONDITIONS AFFECTING GERMINATION

When maturation of the seed is complete certain external conditions are necessary for its further development, the most important of which are a supply of moisture and oxygen, a suitable temperature, and certain conditions of light. In some cases there may also be other special requirements; the seeds of orchids, for example, will not germinate unless a symbiotic fungus is present, without which the embryo remains undifferentiated. The seeds of parasites like broomrape (*Orobanche* spp.) only develop in contact with the roots of host plants, which the seedlings promptly invade.

The role of moisture in germination has been explained on p. 229, and those of the other principal factors are as follows:

Oxygen Supply. In the absence of oxygen seeds can only respire anaerobically, and do not germinate, since the release of energy is not sufficient. This occurs, for example, when they are completely immersed in water, which very greatly reduces the access of oxygen, and it is for this reason that seeds are unable to germinate in waterlogged soils or in soils that are too compact.

Dry seed-coats are surprisingly impervious to gases, and the testas of dry peas and beans are air-tight even at the micropyle. This has been shown by closing the end of a barometer tube with a piece of testa and enclosing the whole in a dry atmosphere. Under these conditions a column of mercury was maintained at atmospheric level, showing that a vacuum had been obtained, and that no air penetrated the closing membrane. As soon as the atmosphere round the testa was moistened it began to take up water, and at the same time the mercury column began to fall, showing that air was passing through the membrane. Peas and beans and perhaps many other seeds are thus hermetically sealed when dry, and one important and rather unexpected effect of the absorption of moisture is to render the seed-coats permeable to gases, including oxygen.

Temperature. Germination will only occur within a limited range of temperatures, but the actual extremes vary a good deal in different species. On the whole, plants of cool and temperate regions have a lower range of germination temperatures than those of warmer countries, the following being characteristic examples:

	Germination Temperatures	
	Minimum	Maximum
Wheat (Temperate) . .	0–5° C.	31–37° C.
Maize (Sub-tropical) . .	5–10° C.	44–50° C.

Above the maximum temperatures soaked seeds are killed, probably on account of heat coagulation of their proteins and enzymes; cooling them below the minimum temperature is not fatal, however, and germination will take place afterwards if the temperature is raised to a suitable level.

Temperatures within the possible range are not all equally good for the purpose, as can be seen by measuring the rates of germination which they allow. This rate can be regarded as inversely proportional to the time taken to reach some definite stage of development, such as the first appearance of the radicle, and when this is ascertained it is found that the best or 'optimum' temperature is somewhere about the middle of the range. The position of the optimum is, however, very indeterminate and depends largely upon the stage of development which is taken as a standard; thus maize germinated at 35° C. puts out its radicle very rapidly, but further growth is very slow, and the seedlings may lag behind others germinated at 15° C. For this reason the idea of optima has been discarded in this connexion.

The unfavourable effect of rather high temperatures upon the later stages of germination is probably due to coagulation of the seedling enzymes, occurring more slowly than at temperatures above the maximum. Temperature also exercises a marked effect upon the rate at which water enters the seed, the higher the temperature the faster being the rate of absorption.

Light. The reasons why oxygen, heat, and moisture affect germination are not hard to understand, but the action of light is much less obvious. In the first place seeds have very different reactions towards it, and may be classified roughly as *light* and *dark seeds*, the former needing to receive a certain amount of light before they can germinate, while the germination of the latter is hindered or prevented by it.

Common species possessing 'light seeds' are the Purple Loosestrife (*Lythrum salicaria*), Willow Herb (*Epilobium hirsutum*), and the buttercup, *Ranunculus sceleratus*. If seeds of any of these are kept entirely in the dark they will not germinate, although a flash lasting no longer than a second will enable many of them to do so. In some cases a temperature above 30° C. will have the same effect as exposure to light, and removal of the testa may also bring about germination, so it seems that the action of light in normal cases may be to make the testa permeable to oxygen and water.

'Dark seeds' are found in *Nigella*, and their germination can be brought about in light, either by removing the seed coats or by exposing them to an unusually low temperature. The explanation of these curious facts has not yet been discovered.

VEGETATIVE DEVELOPMENT

Soon after the seedling of a normal green plant has spread its leaves in the air it is able to photosynthesize, and thereafter lives and grows by the material it assimilates. The development which then occurs is distinguished by two important characters; it continues, though at varying rates, all through the life of the plant, and is restricted to certain growing regions, the meristems. Contrasting with this is the growth of animals, which is completed in youth, and occurs more uniformly, the whole animal keeping more or less the same proportions throughout life.

The stages of growth shown by rapidly dividing cells and those slightly older have been described on p. 122, and these phases are found whether the meristem is young, as in a newly-formed radicle, or old as at the tip of a well-developed root or stem. The cells which have become vacuolated are usually unable to divide even though they are still alive, and once they have reached a certain size they do not increase or multiply any further. As a final stage many of the cells lose their protoplasmic contents altogether, and consist only of the walls formed by them. These represent so much inert matter, which has not even the power of *rejuvenation*, or the resumption of meristematic qualities, which is sometimes shown by adult parenchymatous cells.

The primary meristems of stems and roots give rise to the growth in length of these organs, but their growth in thickness is usually due to the activity of rejuvenated meristems, or *cambia*, which arise in the young parenchyma. The development of the daughter cells of cambia is very similar to the development of cells in the primary tissues, and for the greater part of the plant's life the two processes are going on simultaneously, with the result that it grows in height and girth.

The Measurement of Growth. The processes responsible for growth are exceedingly numerous and complex, and as described above they occur in a number of disconnected positions in the plant. It is even rather difficult to frame a precise definition of what is understood by the term, growth, though in practice one accepts any permanent increase in size as showing its occurrence.

The basis of all vital activity is the active protoplasm of the living cells, so that growth might be defined ideally

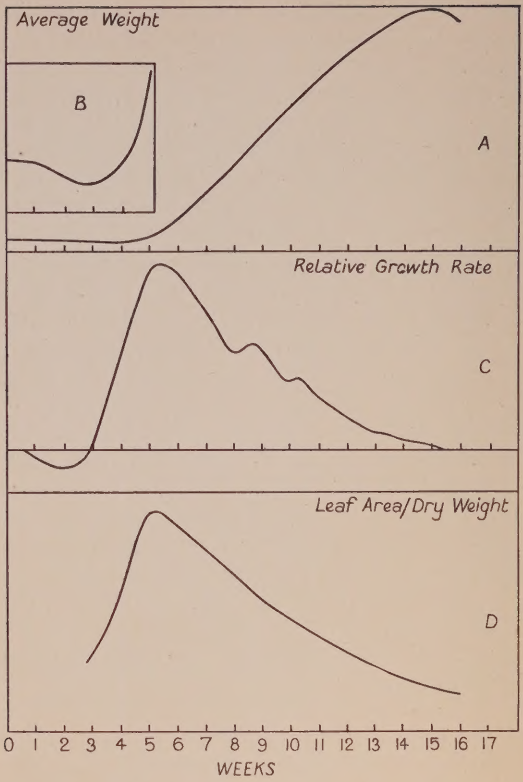


FIG. 71. Graphs illustrating the growth (= increase in dry weight) of the maize plant. *B* shows the initial phase of *A* with the vertical scale enlarged. (*C* and *D* after Briggs, Kidd and West)

as increase of active protoplasm, but this would not coincide with the usual use of the term which includes the formation of inert matter in the woody and other tissues. Moreover, in actual practice it is still impossible to measure the amount of active protoplasm in a complex plant, and in deciding the rate at which growth is going on some simpler standard is necessary. The oldest plan was to measure the increase in length of the plant's main axis, but this had serious disadvantages. It entirely disregarded growth in thickness, which is not necessarily proportional to growth in length, and it would also make plants grown in darkness appear to be growing faster than those kept in light, although actually they are losing matter instead of gaining it. The best system that has been devised up to the present is to measure the rate at which plants increase the amount of their solid material or *dry weight.* This has the great disadvantage that drying kills the plant, so that only one determination can be made with an individual, and large numbers have to be taken to obtain successive values. In spite of this defect the method has been found very valuable, and our modern knowledge of growth is built up mainly upon this basis; older ideas derived from length measurements are generally of less importance.

The Grand Period of Growth. Expressed in general terms the vegetative growth of most plants shows three phases, starting slowly, becoming gradually faster, and finally slowing down again. These three phases cover the whole of the vegetative history of an annual plant, and together are called its *grand period of growth.* In a perennial plant the story is repeated annually with periods of dormancy between the repetitions.

Various attempts have been made to determine the annual growth cycle more precisely, by weighing the dry matter formed at regular intervals. Fig. 71 A shows the average weight each week of samples from a set of maize plants all of which were grown together; similar curves have now been constructed for many other annual plants, and all of them show the same S shape. It should be noticed (fig. 71 B) that during germination, i.e. at the beginning of the curve, the seedling is actually losing weight as shown by the curve's initial dip. After this, for a short time while the plant is still very young, its weight may increase uniformly like a sum of money at continuous compound interest, when the rate of interest is unchanged. This would imply that all the cells in the plant were growing and dividing at equal rates and is only likely to be true as long as the greater part of it is meristematic, and it is, in fact, found that the *relative growth rate*, corresponding with the rate of interest, is not uniform, but varies continually throughout the life of the plant. The relative growth rate is defined as the increase of weight in unit time per gramme of material already formed, and is usually calculated for weekly periods, the average of the weights at the beginning and end of the week being taken as 'the amount of material already formed'. The curve showing the relative growth rate of maize throughout its life-history is set out in fig. 71 C.

As long as the seedling is losing weight the rate is negative, and it does not suddenly leap to its full value when the seedling becomes autotrophic, but climbs to it comparatively slowly. There is thus an initial period, when the young plant is growing slowly, the full relative growth rate only being attained after about five weeks. When this maximum has been reached it is not maintained for long, a decline setting in almost immediately, so that growth becomes slower and slower until finally it ceases with the 'ripening' of the plant.

The fall in relative growth rate is mainly due to the multiplication of cells which are unable to divide, so that although the meristems may continue to form new cells at a more or less uniform rate, the growth of the whole plant becomes progressively slower. Another important factor is the amount of leaf which the plant possesses, since this will have a great influence upon the rate at which the sugars necessary for growth can be formed, and it can, in fact, be shown, that the relative growth rate is highest when the *leaf area ratio* (= leaf area per gramme of dry weight) is at its greatest. There is also a rough correspondence between the two quantities during most of the plant's life (fig. 71 D), that is to say they both increase during the early stages and decrease during the later. The agreement is only rough, because other factors besides sugar formation also affect the rate of growth.

Yield. Another method of measuring the growth of plants can often be employed, and in certain cases is the only one available. This consists of determining the final yield of the plants at the end of their life, this quantity being as it were a summary of growth, since it is affected by every condition that has any influence upon the stages leading up to it. The final weight of the plant may be called its yield, but often, and especially with agricultural crops, the weight of some particular part of it is taken; thus the yield of corn is the weight of grain secured, while that of sugar-beet is the weight of sucrose extracted from the root.

External Conditions Affecting Growth and Yield. It is usually impossible to find out the quantitative effect of external conditions upon the rate of growth during any given week because the rate is affected not only by the conditions during that week, but also by others that have gone before. For instance, a dry spell in spring retards the whole development of most annuals whatever the subsequent weather may be like. It is more often possible, however, to decide the effect of such conditions upon the final yield, and an interesting example of this kind has been worked out with wheat. Records of wheat yields have been kept for many years for individual districts and even for single fields, and by comparing these figures with the weather records the effect of external conditions upon yield can be decided.

The English climate is found to be on the average too wet for wheat, so that every inch of rain above the average reduces the wheat crop. The amount of the reduction depends, however, upon the time of year at which the rain falls, the greatest damage being due to rain in January and July, and the least to rain in May. In a certain Hertfordshire field the harvest is on the average reduced by as much as a bushel and a half per acre by every inch of January rain above the average for the month, but in East Anglia, where the soil is lighter and the average rainfall lower, much less harm is caused.

Yield is also affected by the amount of nutrient material in the soil, and the quantitative effect of each substance depends not only on its own concentration, but also upon the concentrations of the other essential materials. Thus additions of potassium salts to a soil will not increase its fertility if nitrogen is deficient, and the extent to which potassium can profitably be added is decided by the available quantities of nitrogen and other essentials. In general terms it may be said that if a single nutrient, or other factor of the environment, is increased by equal steps without altering any others, the increase in yield becomes less with each increase of the factor.

VEGETATIVE REPRODUCTION

Pieces of stem or root cut from their parent plant and fixed in moist soil are able to regenerate new organs, and grow into independent plants. Since this method of reproduction does not involve any specialized cells or organs, it is called *vegetative reproduction* to distinguish it from *sexual reproduction*, involving the fusion of special cells (gametes), and *asexual reproduction*, in which a single special reproductive cell or group of cells is formed without a prior fusion. Sexual reproduction in higher plants leads to the formation of an embryo inside a seed, asexual reproduction occurs in the formation of the spores, i.e. pollen, and megaspores, and various kinds of vegetative reproduction occur also.

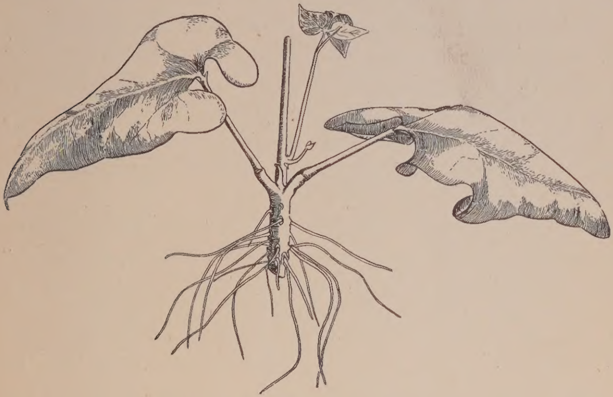


FIG. 72. A CUTTING OF *Phaseolus multiflorus*

i.e. a piece of stem cut away at top and bottom and kept standing in water. A cluster of young roots has been formed, bursting through the swollen tissues at the lower end of the piece of stem. The axillary shoot has also developed owing to the cutting away of the leader

Propagation by means of 'cuttings' is frequently used by gardeners to preserve choice varieties. In this way stems, roots, and even leaves (figs. 72 and 73) can be made the basis of new plants, which show all the characters of the original, since there is no mixing of strains as in sexual reproduction. It is said that continuous vegetative reproduction sometimes eventually causes loss of vigour, and greater susceptibility to disease, a well-known instance being that of the garden hollyhock (*Althea rosea*), which was cultivated for many years from cuttings. The plants thus produced were very liable to the attacks of the rust, *Puccinia malvacearum*, which even threatened to extinguish the species entirely until propagation from seed was resorted to. Seedling hollyhocks and the adult plants into which they grow generally appear much more resistant to the attack of the fungus than plants produced from cuttings, but there are, on the other hand, numerous species which have been kept in cultivation for many plant generations by vegetative means alone, and which still show no sign of deterioration.

Natural vegetative reproduction may be brought about by organs, which have become swollen owing to the precipitation within them of large quantities of reserve substances; thus potato tubers and crocus corms are swollen segments of stem containing starch. In other plants organs showing no obvious swelling may be effective, thus the underground stems of twitch grass (*Agropyrum repens*) may give rise to new plants from any node, and bramble (*Rubus fruticosus*) stems may root and form new plantlets wherever they touch the ground.

Very often this method of reproduction is associated with a previous period of dormancy and so affords a second means of hibernation. The principal physiological difference between dormant seeds and vegetative organs in a similar condition is that the latter do not show the dryness characteristic of seeds, but other differences exist also. As an example, the dormancy and rejuvenation of a potato tuber are described below, and may be compared with those of a seed of the same species described on p. 232.

The tuber is usually formed at the tip of an underground stem, though almost any side shoot can be forced into tuber form by keeping it in darkness and enclosing

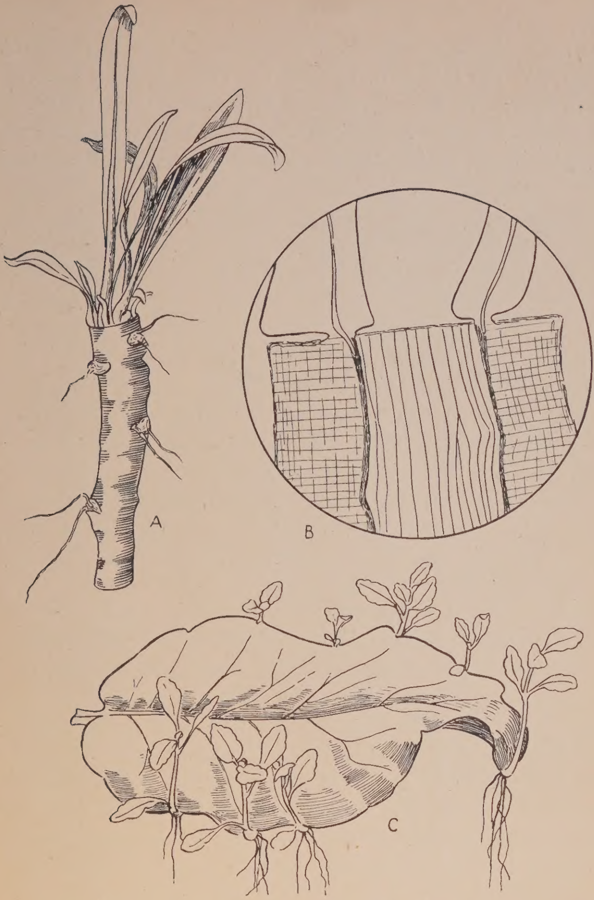


FIG. 73. ROOT AND LEAF CUTTINGS

A. a piece of *Anchusa italica* root, which has been planted in damp soil. A cluster of leaves has been produced at the upper end. *B*. enlarged vertical section showing the origin of the new leaves from the cambium shown by dark shading; wood, vertical shading; cortex, etc., cross hatched. *C*. leaf of *Bryophyllum calycinum* which has been kept on damp fibre. Young plantlets are forming at the notches round the edge of the leaf. (*A* and *C* somewhat reduced) it in a moist atmosphere. Simultaneously with the swelling, starch is laid down in all the cells, and by the time the tuber is mature numerous large grains are contained in each of them, and form the bulk of the organ's dry weight. Fats, which are the principal reserve of the seed, are almost entirely absent from the tuber, and proteins are limited to the strip of cambium and phloem and to the corky skin (fig. 74); a trace of amino-acid may also be present. In the autumn sugars are very scarce or entirely absent, but as the tuber lies in the ground, or in storage, they gradually appear in the tissues immediately behind the 'eyes', or groups of buds. In spring the tuber germinates by the development of one or more buds, which first give rise to stems, roots being formed soon afterwards from their lower segments. The apex of the young stem shows an abundant formation of protein (fig. 74 B), and

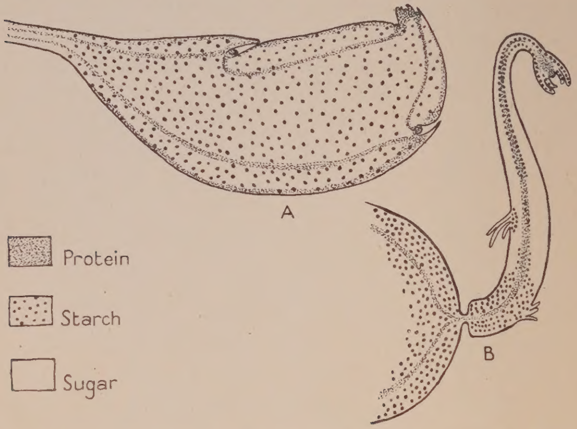


FIG. 74. POTATO TUBER (A) AND SPROUT (B) SHOWING THE DISTRIBUTION OF RESERVES

Shading as in fig. 69 for comparison. (After de Vries, modified) behind this there is a starch region, replaced in its turn by a sugar region, and another region of starch at the base. These tissues and reserves are formed at the expense of the starch in the tuber, which disappears first of all at the centre, the potato even becoming hollow, while there is still a large amount of starch in the outer cells. The evacuation goes on long after the young sprouts have started to assimilate and even to a late stage of the plant's life, when many young tubers are in course of formation.

THE REPRODUCTIVE PHASE

Sooner or later vegetative development is interrupted by the onset of a reproductive phase in which seeds or other special bodies are formed. The time at which this takes place depends upon a large variety of factors, many of them internal or inherent in the plant. The majority of plants in temperate regions set their seed at the end of the summer after a period of active vegetative growth, but there are many perennials, which flower before their leaves unfold for the year, and thus commence their reproduction at the beginning of the active season. Differences such as this must depend upon the different natures of the plants, and how they are brought about is beyond our present knowledge.

The amount of carbohydrate material in plants is always greater than the amount of nitrogenous material, and it has been observed that at the time of flowering the excess becomes particularly great. This is usually expressed by saying that the carbon/nitrogen ratio is high, and if it is kept down for any reason flowering is usually delayed or even prevented.

External conditions may affect the period at which flowering begins to a limited extent; thus a soil which is rich in nitrogen will tend to keep down the carbon/nitrogen ratio of plants growing upon it by increasing their nitrogen, and this in turn inhibits flowering. Converse or similar effects are caused by other nutritional substances and also by meteorological conditions, such as lack of moisture, which often provokes early flowering. One of the most important factors is light, particularly in regard to the length of the periods for which it is applied. Many plants come into flower at a much earlier date than usual if the daily illumination is increased artificially, whereas others respond in this way to abnormally short illuminations. By suitable adjustments of the lighting conditions plants which normally flower only in their second season can be made to flower after a few months, and conversely the flowering of annual plants may be postponed indefinitely.

EXPERIMENTAL WORK

Exp. 66. Correlation

Sow some broad beans in fine soil near the surface of a five-inch flower-pot. When the first leaf and the internode above have developed, cut off the tip. The bud in the axil of the leaf will then begin to grow, and if this is removed in its turn the buds in the axils of the cotyledons will start to grow out forming a pair of shoots. In each case the lower bud is unable to develop while the one above it is intact. This may be shown by leaving some of the plants untouched.

Exp. 67. Reserve Substances in Mature Seeds

Cut half a dozen thin slices of castor-oil, lupin, preferably *Lupinus pilosus*, and bean seeds. It will be necessary to strip away the hard testa of the castor-oil seeds before cutting. Place the sections in nine separate watch-glasses, three of each species and test each seed for

a. starch by adding iodine solution.
b. cellulose by adding chlor-zinc-iodine.
c. fats by adding Sudan III.

Lay the watch-glasses in a regular order upon a sheet of white paper and notice the comparative intensities of the colours produced indicating roughly the amount of material present. The results will be as follows:

	Castor-oil	*Lupin*	*Bean*
Starch	None	None	Much
Cellulose	Little	Much	Little
Fats	Much	Little	None

Test the same seeds for sugars with Fehling's solution (*see* **exp. 1**).

Exp. 68. UPTAKE OF WATER BY SEEDS

a. Count out ten dry peas and weigh them to the nearest centigram. Then determine their volume by dropping them gently into a narrow measuring cylinder containing sufficient water to cover them, and noting the rise in level of the water. Allow the seeds to soak for 24 hours and then measure their volume again. Dry their surfaces gently with blotting-paper, or filter-paper, and then weigh. Both weight and volume will have increased owing to the uptake of water.

b. Weigh five seeds of *Canna indica* to the nearest centigram and then leave in a beaker of water for a week. Carefully dry the surfaces with blotting-paper and weigh again. There will be little or no increase of weight. File a hole in the testa of each seed and soak for one or two days longer. On weighing once more there will be a marked increase due to the absorption of water through the hole in the impervious testa.

For other experiments on germination see **Exp. 44.** The Effect of High Temperatures, p. 148, and **Exp. 31.** Loss of Weight during Germination, p. 118.

Exp. 69. THE EFFECT OF LIGHT ON SEEDLING DEVELOPMENT

Sow half a dozen oat seeds near the bottom of a glass box with about 6 inches of fine soil above them. Place the seeds against one side of the box so that light will easily reach them, and place in a bright window with the box sloping gently backwards. This will ensure that the seedling shoots grow close against the glass. Set up a second lot of seeds but keep them in the dark. The first set will grow as shown in fig. 68, but the second set will reach the surface without difficulty.

Carry out a similar experiment with peas.

Exp. 70. THE GROWTH OF EPHEMERALS

The principal features of vegetative growth may be observed by growing quick-developing ‘ephemerals’, such as Chickweed (*Stellaria media*) or Speedwell (*Veronica* spp.), in pans of fine soil. Ripe seeds collected early in the season should be sown, not too thickly, and covered with a thin layer of soil and sand, and kept well watered.

Secondary thickening is very slight in these species, so that a fairly accurate estimate of growth may be made by measuring the length of important organs. Determine (*a*) the weekly increase of length of the main stalk, or stalks, and (*b*) the length of individual internodes. The latter series of measurements will show where growth is most rapid. Is the rate of growth fast or slow at the onset of flowering?

Exp. 71. VEGETATIVE REPRODUCTION

a. Stem Cuttings. Take cuttings of runner beans or willow twigs. Cut a fresh surface under water and keep with the cut ends in a vessel of water or wet sand. Put the whole into a large wooden box with a sheet of glass over the top to maintain a moist atmosphere, and keep warm. After a week or a fortnight new roots will have formed at and near the cut surfaces. If now planted out in soil the cuttings will continue to grow.

b. Root Cuttings. Pieces of the thick root of dock, dandelion, or *Anchusa*, 2 or 3 inches long, may be planted in wet sand and kept in a moist atmosphere as before. New shoots and fine rootlets will develop, and a fresh plant be thus started.

LIST OF SPECIAL REAGENTS AND METHODS OF PREPARATION

Alcohol. Required strengths may be obtained by diluting laboratory methylated spirits (= 95 per cent. alcohol).

Aniline chloride. Make up a saturated solution in distilled water, filter and acidify with a few drops of hydrochloric acid.

Aniline sulphate. Make up as aniline chloride, but acidify with sulphuric acid.

Baryta water. N/10. Dissolve about 50 gm. of barium hydroxide and 15 gm. of barium chloride in 500 c.c. of boiling distilled water. Allow the solution to cool with a tube of soda lime corked into the neck of the flask. The excess barium hydroxide will crystallize out, and the clear fluid may be siphoned off into a second vessel, from which carbon dioxide has been removed, without allowing the solution to come into contact with the outer air. Its strength will be about 0·35 N. Add about a litre of freshly boiled distilled water, which has cooled under soda lime, and standardize with N/10 hydrochloric acid, using phenolphthalein.

Brom-cresol purple. Solutions in alcohol may be obtained ready for use.

Calcium chloride. Dissolves readily in water. Molar solution (M) = 111 gm. per litre.

Caustic soda. Strong solution = 40 per cent. When dissolving in water keep well stirred, or the heat evolved may crack the vessel.

Chloral hydrate. Dissolve 160 gm. of chloral hydrate crystals in every 100 c.c. of water.

Chlor-zinc-iodine. (Schultze's Solution.) Dissolve 110 gm. of zinc in 300 c.c. of hydrochloric acid and evaporate to half the volume. During the evaporation add a little extra zinc to make sure that all the acid is neutralized. These operations are best carried out in the open. Then dissolve 10 gm. of potassium iodide in the least possible quantity of water and add 0·15 gm. of iodine crystals. Mix the two solutions and if turbid filter through glass wool. Keep in a tightly stoppered bottle in the dark.

Cobalt chloride paper. Cut strips of filter-paper about one inch by two, and dip into a 5 per cent. solution of cobalt chloride. Dry in an oven at 100° C. and keep in a desiccator.

Fehling's solution. Must be made up as two separate solutions which are mixed immediately before use.

Soln. A. 70 gm. of crystalline copper sulphate in a litre of distilled water.

Soln. B. 350 gm. of Rochelle salt (sodium potassium tartrate) and 100 gm. of caustic soda in a litre of distilled water. Mix equal quantities of A and B.

Glycerine, dilute solution = 5 per cent.; prepared by making up 5 gm. of pure glycerine to 100 c.c. with distilled water.

Hydrochloric acid, N/10. Dilute 11·3 c.c. of concentrated acid (sp. gr. = 1·16 at 15° C.) to a litre. The resulting solution will be roughly decinormal. For quantitative work it must be standardized against a solution of pure sodium carbonate made up by weighing.

Iodine, solution in alcohol. Made by shaking up the crystals in alcohol until a deep golden brown is obtained.

Iodine, in potassium iodide. Make a strong solution of potassium iodide; add some crystals of iodine and shake up from time to time. The solution as used should be a dark golden brown.

Lime-water. Shake up an excess of freshly slaked lime with distilled water in a Winchester. Allow to stand until clear and then siphon off the liquid into a second Winchester, which has been freed from atmospheric carbon dioxide. Can also be obtained from druggists.

Millon's reagent. 15 gm. of mercury are dissolved (in a fume cupboard) in 43 c.c. of strong nitric acid. Dilute with 80 c.c. of distilled water and filter after two hours. Can be obtained from dealers ready for use.

Phenol phthalein. Dissolve 1 gm. in 100 c.c. of methylated spirit.

Phloroglucin. Make a saturated solution in alcohol and gradually add strong hydrochloric acid until precipitation begins.

Pyrogallol, alkaline solution. Dissolve 28 gm. of pyrogallol in 100 c.c. of distilled water, and 50 gm. of potassium hydroxide in a second 100 c.c. Mix the two solutions immediately before use.

Schimper's solution. 160 gm. of chloral hydrate in every 100 c.c. of distilled water. Colour by adding a few drops of strong iodine solution.

Sodium picrate paper. Dip strips of filter-paper into 1 per cent. picric acid, and allow to dry in air. Just before use moisten with 10 per cent. sodium carbonate.

Starch solution. Mix 1 gm. of dry starch into a suspension in a little cold distilled water. Bring 200 c.c. of distilled water to the boil in a beaker, and pour in the suspension, stirring all the time. Continue to boil for about a minute and then allow to cool. If the solution is at all lumpy a part of it may be filtered while still hot. The filter-paper soon becomes clogged, but the clear filtrate contains sufficient starch to give a deep coloration with iodine.

Sucrose. Commercial granulated sugar is extremely pure and may be used. For a molar solution dissolve 342 gm. and make up to a litre.

Sudan III, alcoholic solutions may be obtained ready for use

INDEX

The more important references are given in heavy type

acetic acid 11
acidity 94, 136, 138
after-ripening 229
albumin 71
alcohol 5, 102, **111–14**
amino acid 69, **73–6,** 110, 177, 227, 234
ammonia 77, 85–7
amylase 141
amyloid 53
anaerobe 103
anthocyanin 54
argon 8
atmosphere 5, 8–17, 24, 30, 32, 105, 152
autolysis 127

bacteria 7, 10, **32–5, 80–90**
Beggiatoa 33
bicarbonate **11–12,** 21
butyric acid 11

calcium 8, 11, 142, 184, 185
carbon 8, 9, 45, 69, 232
carbon dioxide **8–13,** 17, 20, 26, 29, 31, 33, 92, 94, 102, 111, 228
carotin 24
catalyst 121, **137**
catechol 106
caustic soda 17
cell 4, 5, **121–8**
— wall 17, 20, 122, 125
cellose 53, 55
cellulose 10, 31, 45, **52–3,** 55, 126
cereal 57
charcoal 138
Chlorella 32
chloroform 128
chlorophyll 8, **22–7,** 28, 29, 31
chloroplast 13, 18, 20, 28, 29, 209
chromatic adaptation 22
clay 129, 152
clinostat 199
coagulation 133, 136
coenocyte 125
co-enzyme 140
coleoptile 206, 211, 231
collodion 132
combustion 9
compass plant 215
Congo red 132
corm 246
critical concentration 59
cuticle 23, 154
cutin 53, 164
cutting 245
cytase 141
cytoplasm 13, 122, 126

denitrification 88–90
development 1
dextrin 58
diastase 59, 141
diffusion **13–17,** 20, 131, 180
dipeptide 69

edestin 71
embryo 95
emulsin 141
emulsion 3
endodermis 162–4
environment 3–5
enzymes 58, 72, 79, 94, 106, 127, 133, **137–41**
epidermis 13, 20
erepsin 138, 141
ethylene 128
etiolation 205
evaporation 5, 152–4
excitation 195

fat 32, **64–5,** 227, 232
fatty acid 64, 140
Fehling's reaction 47
fermentation 9, 11, 101, **111–15**
flavone 54
flower 205, 226
formaldehyde 27, 73
formic acid 11
fructose 29, 45, **47,** 55, 58, 112, 113
fruit 95, 128
fungi 32, 78
furnace gases 11

galactose 53, 112
generation time 223
globulin 71
glucose 29, 31, **45–9,** 55, 58, 63, 98, 103, 112
glucoside **53,** 61
glutelin 71
glycerine 64, 140
glycine 69, 75
glycogen 53, 113
glycolysis 92, 102, 103, 113
guard cell 13, 17, 18, 60, 156
gum 46

heat 108, 138
— of combustion 31
hormone 201
humus 12, 78, 152
hydrogen 8, 11, 33, 45, 69, 105, 232
— ion 137, 184
— peroxide 107
Hydrogenomonas 33
hypocotyl 197, 206

imbibition 133
induction 195, 200, 212
— period 208
inhibition 225
inulin 53
invertase 138, 141
iodine 21, 53
ion 128
iron 8, 34, 142
isomerism 48

leaf 4, **13–20,** 32, 95, 205, 225, 243, 245
Leptothrix 33, 34
leucine 31
leucoplast 127
light 8, 18, **21–4,** 28, 31, 32, 73, 86, **204–216,** 238, 250
lignin 53
lipase 140, 141

magnesium 8, 25, 26, 28, 142
malic acid 109
maltase 141
maltose 45, 46, **50,** 55, 58
mannose 45, 47, 53, 58
materialist 2
membrane 131
— semipermeable 158
meristem 76, 95, 122, 238
mesophyll 13, 17, 154
metabolism 61, 63
metaprotein 70
methane 11
methylene blue 106
monosaccharide 45
mucilage 46, 53, 170
mycorrhyza 78

nitrate 73, 75, 77, 88
nitrification 85, 89, 90
nitrite 77, 86
Nitrobacter 33–5
nitrogen 8, 25, 35, 69, 73, **77–90,** 232
Nitrosomonas 33–5
nucleo-protein 72, 76, 227, 234
nucleus 122, 124

oil 64, 134
olive oil 31
organic acid 11, 46, 75
organization resistance 127
osmosis 2, **158**
osmotic pressure, 18, 60, **158**
oxidase 106
oxidation 89, 90, 93, **105**
oxido-reduction 105
oxygen 8, 11, 25, 30, 45, 69, **100–11,** 232, 236
oxygenase 107, 141

palisade 13, 209
palladium 105
pentose **45,** 53, 54
pepsin 138, 141
peptide linkage 70
peptone 70, 234
peroxidase 107, 141
phaeophytin 26
phenol 107
phloem 181
phosphate 235
phosphorus 8, 28, 142
plastid 13, 122
poison 61
polarized light 49
polypeptide 70, 234
polysaccharide 45, **50–3,** 100
potassium 8, 72, 136, 141, 180
potato 18, 232, 246
prolamin 71

protein 32, **69–76,** 179, 233, 248
proteose 70
prunase 141
pulvinus 195, 206, 215
putrefaction 90

radicle 230
reductase 141
rejuvenation 239
relative growth rate 242
reproduction 3, **244–50**
respiration coefficient 100, 110
respiratory index 95
root 13, 156, 197, 207, 211, 225, 245
— hair 156, 179
— pressure 164

sand dune 5
sea 5, 24
seaweed 22, 46
seed 4, 64, 71, 95, 102, 108, 109, 170, 180, **225–38**
— coat 229, 236
seedling 96
sieve tube 181
silicic acid 128
sleep movement 205
soil 4, 5, 8, 9, 12, 35, 83–90, 152
— respiration 9
— solution 182
— water 4, 152
solution 17
solvent 17
souring 115
Spartina Townsendii 5
spectrum 22, 23–5
Sphagnum 5
sporangiophore 206
starch 19, 29–31, 45, **51–2**, 55, 58–61, 63, 129, 178, 227, 232, 248
— leaves 60
starvation 98–101, 110
stem 13, 202, 211
stoma **13–19,** 30, 56, 60, 154–6, 174
succulent 109
sucrose 29, 31, 45, **49,** 55–60, 63
suction pressure **156,** 229
sugar beet 49, 57
— cane 49, 57
— leaves 60
sulphur 8, 33, 34, 142
— bacterium 33–4
sulphuretted hydrogen 33, 138
surface tension **134,** 166
swarm spores 208
symbiosis 84

temperature 5, 21, 28, 94, 237
testa 229, 236
Theophrastus 1, 82
tissue 124
transpiration 32, **153–6,** 170
— stream 139, 154, 181
triolein 110
tropism 196
tuber 246
turgor 161, 215

ultramicroscope 130

vacuole 122, 135
viscosity 135
vitalist 2
volcano 9

xanthophyll 24
xylem 164, 167

yeast 5, 106, 111, 222

zymase 95, **113,** 139
zymin 114

PRINTED IN GREAT BRITAIN AT THE UNIVERSITY PRESS, OXFORD
BY JOHN JOHNSON, PRINTER TO THE UNIVERSITY

BINDING LIST JUN 15 194

Bo
J
263385
Author James, William Owen
Title An introduction to plant physiology.

University of Toronto
Library

DO NOT
REMOVE
THE
CARD
FROM
THIS
POCKET